A STUDY OF SOME ASPECTS OF THE METABOLIC PROFILE OF GRAZING DAIRY CATTLE IN NEW ZEALAND

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ABSTRACT

Groups of cattle on three dairy units at Massey University were sampled on a monthly basis for a period of twelve months to collect data on 11 blood parameters that would provide the basis for a metabolic profile for grazing dairy cattle in New Zealand. The parameters selected were those initially in the 'Compton Profile' i.e. haematocrit, haemoglobin, total protein, albumin, urea nitrogen, glucose, sodium, potassium, magnesium, calcium and inorganic phosphate.

Comparison between the Massey and the U.K. results revealed that haematocrit and haemoglobin values were lower and serum total protein, urea nitrogen and glucose values higher than the comparable figures for the U.K. and almost all parameters for the New Zealand data were more variable. Possible reasons for differences were discussed.

The design of the investigation permitted the estimation of a number of sources of variation, namely season, lactation and age. To obtain additional information a further herd was sampled for another year and in another location. Seasonal variation occurred with most parameters although this was minimal with sodium and potassium. The seasonal variation in haematocrit and haemoglobin followed a consistent pattern with high values in winter and summer and low values in autumn and spring. Urea nitrogen values showed marked changes which followed the variation in pasture protein content but which were apparently modified by the amount of feed offered. In the case of other parameters seasonal change appeared to be minor and/or inconsistent; nevertheless it could at times be important, e.g. low serum magnesium in the spring in one herd only.

Stage of lactation appeared to have little influence on the values recorded except at times of peak lactation when nutritional insufficiency was also present. Inorganic

phosphate and calcium showed a decrease with age and globulin showed an increase; the extent of these changes was relatively small. Age had minimal influence on all other parameters measured.

In an attempt to define other factors contributing to the variation observed an additional two investigations were carried out: the first where sets of identical twin cows were sampled daily for three consecutive days each month for thirteen months; the second where two hourly samples were collected for a 12 day period from cattle which were housed, and fed and milked on a rotation which allowed the effects of diurnal variation (if any) and the influence of these two variables to be separated.

Monthly changes in the values of the parameters, which represent the combined effects of season and lactation, was an important source of variation in all cases but daily variation was found to be relatively unimportant. Significant genetic effects were observed with haematocrit and haemoglobin, to a lesser extent with urea nitrogen, total protein and albumin, and to a minor extent with glucose, potassium,calcium and inorganic phosphate.

Significant diurnal rhythms were observed with sodium, calcium and inorganic phosphate, with the latter two tending to move together. Time since milking was relatively unimportant as a source of variation.

Time since feed was first offered was an important source of variation in the case of haematocrit, haemoglobin and inorganic phosphate while the amount of feed consumed was important with haematocrit, haemoglobin, total protein, albumin and calcium.

Despite the efforts that were made to standardise procedures throughout the entire investigation, and to partition the total variance to a number of likely sources, the residual variation remained high. Further investigations are warranted

to define further the factors that contribute to this residual variation before the potential of the 'metabolic profile' as a diagnostic tool can be properly exploited.

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CHAPTER I
INTRODUCTION

The use of the term 'Metabolic profile' in a veterinary context has only come to the fore in recent years whereas the technique, although not often referred to by that name, has been in use in the medical field for a much longer period.

No simple definition exists which adequately defines a metabolic profile as it has been applied in veterinary medicine: methods used and analyses performed have varied according to the problems of the area in which the work has been carried out and the interests and objectives the particular worker(s) had in mind when initiating the investigation. The original profile described in cattle (Payne *et al.*, 1970a) consisted of a series of blood assays on a number of samples collected from the herd so that an evaluation of the results would provide a realistic measure of the health of that herd.

For this purpose the technique used has been to divide the herd into categories such as those in peak lactation, mid-lactation and non-lactation and to sample a number of animals (usually seven) from each category. Information gained from the assays performed has been used to assess the adequacy of nutrition, to determine the cause of subnormal productivity and reproductive performance, and as an aid in the elimination of some clinical diseases. While it was appreciated that samples other than blood could provide more effective information concerning some of the problems being investigated e.g. saliva for the sodium status, the intention of using blood samples to provide the basic information had the virtue of simplicity; collection, handling and analytical procedures could be easily standardised using this approach and a relatively wide range of parameters measured. For these reasons, and the fact that such an approach could readily be applied by veterinary practitioners in the field, the author accepted the blood sample as an appropriate base from which the investigation

described in this thesis could be conducted. Furthermore, because husbandry conditions in New Zealand varied widely from those in many other parts of the world, with cattle grazing competitively at high stocking rates for food which varies widely in both quantity and quality, the importance of establishing a profile for these conditions seemed fully justified.

The accepted baseline for normality in the U.K. for a particular parameter was that the herd mean fell within an established mean and two standard deviation range (Payne *et al.*, 1970b). This established mean was derived from accumulated metabolic profile results at that time. While obtaining this type of data for New Zealand conditions was clearly important it was equally necessary to learn how much an animal varied about an 'established mean' as part of its normal healthy living pattern since this would have to be taken into account in any interpretation of a profile result. Season, age and the effects of stage of lactation seemed major sources of variation requiring investigation.

The initial stages of the project involved following animals selected for a range of ages and calving dates throughout a twelve month period. Obtaining a mean for all samples eliminated the effects of the sources of variation referred to above while calculation of the variance about the mean from these sources gave an estimate of the influence each exerted on the various parameters. This information was obtained for a group of local herds (the three Massey University dairy units) and for a further herd in a different geographical location (the Awaroa herd).

When the data had been assessed two further investigations were undertaken in an attempt to isolate other factors that could have been contributing to the variation that was observed with each of the eleven parameters being measured. The first of these involved sampling pairs of identical twins using a design that permitted evaluation of the extent of the genetic control of each parameter as well as monthly,

daily and individual animal components of the variation and some of their interactions. Following this a further group of identical twin cattle were housed, sampled, fed and milked on a schedule that enabled results to be analysed so that the effects of feeding, milking and the twenty four hour diurnal cycle could be separated and the nature and extent of the variation due to these sources assessed.

The project described in this thesis was therefore undertaken in four parts with the results and discussion for each being dealt with as a separate chapter. A final section brings together in summary form the conclusions which were reached as a result of the total study and finishes with some comments as to further areas that need to be explored before the full potential of the metabolic profile in dairy cattle will be realised.

The appendices contain details of methodology and analyses not appropriate to the main body of the text together with a copy of a paper on "A continuous metabolic profile of grazing dairy cattle over a one year period," which was presented at the 9th International Congress on Diseases of Cattle, Paris 1976 during the course of this investigation.

CHAPTER II

REVIEW OF LITERATURE

A. THE DEVELOPMENT AND USE OF METABOLIC PROFILE TESTS IN CATTLE

The metabolic profile in human medicine, both to assess the health status of a population, and as a routine screening test for individual patients admitted to hospital (Williams *et al.*, 1970; Harris *et al.*, 1970; Cotlove *et al.*, 1970; O'Kell and Elliott, 1970), has been in use for some time. This has not been the case with cattle however and although the blood testing of these animals has been practised for many years, the use of a number of tests carried out on a single blood sample to assess the health status of a clinically normal cow has become economically viable only with the advent of automated analytical equipment.

Several attempts have been made to establish normal values for a number of the blood components in dairy cattle (Fisher, 1960; Tashjian *et al.*, 1968; Lane *et al.*, 1968). These authors have tended to review earlier work, publish their own results and discuss the reasons for variations from the previous values reported. Almost invariably only small numbers of animals were involved in the samples, or numbers were not cited in these communications. Furthermore, although some of the conditions under which samples were collected and evaluated were stabilized, a number of factors such as the level of nutrition, proximity to calving, age, breed, time of day or year and housing conditions, all of which could influence the result, were either not considered in the paper or insufficient evidence was given to allow the reader to deduce these. Lane *et al.* (1968) produced the most comprehensive information by testing 236 cows at three monthly intervals; the range of parameters examined however was limited and included only phosphorus, magnesium, calcium, sodium and potassium.

The first time a metabolic profile was carried out in cattle, similar to that referred to in the medical literature, was by Payne and co-workers in the United Kingdom; the original communication appeared in a paper presented at a physiology conference (Payne *et al.*, 1970a). A subsequent paper on the same study was published (Payne *et al.*, 1970b) which caught the interest of the veterinary profession, and since then a number of reports of studies by this group of workers have appeared, many of which have been reviewed in a paper by Rowlands (1980a). In the initial study analyses were carried out for haematocrit, haemoglobin, total serum protein, albumin, and by arithmetic difference globulin , urea nitrogen, glucose, sodium, potassium, magnesium, calcium and inorganic phosphate. The haematocrit was omitted from later studies because of the inability, in cattle, to automate the procedure, and also because of its expected close correlation with the haemoglobin level (Payne *et al.*, 1974), while a copper analysis was added because of a possible association with reduced fertility (King, 1971). Little statistical data for support of this latter contention has been published as yet and, in fact, some evidence has been published to the contrary (Rowlands *et al.*, 1977b). Copper estimations now appear to be aimed at detecting cattle suffering from a low blood copper with a view to correcting this on the assumption that such cattle produce at a sub-optimal level.

Rowlands *et al.* (1974a) added serum iron to the profile to obtain an understanding of the causes of anaemia. Total iron binding capacity has now been added (Kitchenham *et al.*, 1977) together with percentage iron saturation as these provide more useful information than serum iron alone. From their original work Payne *et al.* (1970a) indicated that the metabolic profile test could be used as a measure of input - output balance and that dietary changes could be implemented if the information obtained indicated that this was desirable. As a consequence the concept of 'Production disease' arose, a term used to describe a situation where productivity became sub-optimal due to excessive metabolism of an animals own body reserves. The aim of the test was to detect this imbalance of

input - output before it had serious long term effects (Payne, 1972a).

Seasonal differences had already been reported to influence some serum and blood components prior to work on metabolic profiles (Clawson, 1914). It had also been indicated that seasonal variation could be fairly large but of a similar pattern in successive years (Payne *et al.*, 1967). In a paper by Rowlands *et al.* (1974a) seasonal differences were followed and discussed but the assumption was made that the within - herd between - animal variance was minimal and there was no need to follow the same animal throughout the course of a full year. In view of the method adopted for obtaining information i.e. the sampling of seven high - yielding, seven mid - lactation and seven non - lactating cows, following one animal through a whole year was not possible. The validity of the results thus depended on the accuracy of this original assumption. Certainly it had been demonstrated that the within - herd was far less than the between-herd difference (Payne *et al.*, 1970b). Other workers have also followed seasonal trends by recording the mean of a large number of animals for a number of parameters (Ross and Halliday, 1976). Although seasonal trends were present no attempt was made by the authors of either paper (Rowlands *et al.*, 1974a; Ross and Halliday, 1976) to suggest variations in the interpretation of blood values, either individually or as part of a profile, according to the date of the sampling.

In the original report by Payne *et al.* (1970a) another aspect referred to was the relationship between profile measurements and growth rate; calves which had a high mean serum glucose, a stable serum albumin, a high stable serum calcium and a low serum potassium were found to grow more rapidly than those which did not. These relationships had become characteristic by 2 - 3 months of age. Later papers have been published which further explore the relationship between metabolic profile findings and growth rate (Rowlands *et al.*, 1974a; Farver *et al.*, 1980), management systems (Kitchenham *et al.*, 1977; Manston *et al.*,

1977), breed (Rowlands *et al.*, 1977a), diet (Manston *et al.*, 1975; Treacher *et al.*, 1976), lactation and pregnancy (Rowlands *et al.*, 1975), and reproduction (Rowlands *et al.*, 1977b, McClure and Payne, 1978). Most have followed from trials in which the profile has been used as a research tool to investigate differences between groups of cattle subjected to different experimental treatments rather than from day to day field situations. Experience has also accumulated from field situations (Stevens, 1975; Smyth, 1976) so that a greater understanding and better interpretation of metabolic profiles is developing from their continuing use.

According to Blowey (1972) and Blowey *et al.*(1973) the input-output balance may be effectively measured by a smaller range of tests than those referred to by Payne *et al.* (1970a). They record a profile carried out in a different manner, namely by selecting at each bleeding cows which had similar (or the same) post-calving intervals. This eliminated the effect of lactation on the profile but took no account of seasonal influences. Evaluations were made of glucose, urea nitrogen and albumin levels only. The cattle were fed various diets and a close correlation between diet and some of the parameters, especially glucose, was recorded in a proportion but not all of the herds under study. Blowey *et al.* (1973) realized that not every case of low energy intake resulted in lowered blood glucose, nor did every case of lowered blood glucose respond to increased energy in the diet, and they questioned the relationship between glucose levels and energy balance. In a subsequent paper Parker and Blowey (1976) stated that ".... within the nutritional ranges encountered, the levels of selected blood components did not show a constant relationship to nutrient balance or potential fertility". In this same article they went on to make what is perhaps a very relevant statement about the use of the metabolic profile test in general i.e. "the technique is more appropriately regarded as an aid to the conventional approach involving the examination of feeding systems and feedstuffs, herd records, management and clinical conditions." The inference clearly is that such test are diagnostic aids and not diagnostic substitutes.

Thus Michel and Perrier (1977) used a standard Compton profile for finding solutions to problems not responding to a conventional diagnostic approach. After establishment of local normal values they applied it to four herds and by correcting deficiencies in nutrition resolved problems concerned with decalcification, acetonaemia, mastitis, lowered fertility and footrot. Not all attempts to use the test in this manner have been successful however, and Reyes and Currell (1974), using the profile on cattle in Rhodesia had an unreliable and unsatisfactory profile result. They found transport of samples a problem, possibly associated with the high environmental temperature, and noted considerable variation in the results with the manual testing methods that were used.

Kronfeld (1972), in discussing the selection of parameters for testing in a profile, stated that with automated systems, low cost and convenience were often selected before relevance. For estimation of energy balance, and to recognize the prodromal stages of metabolic diseases, he suggested that instead of Payne's "Compton Profile" the following be measured: calcium, magnesium, inorganic phosphate, glucose, free fatty acids, aceto-acetate, betahydroxybutyrate, acetate, total protein, albumin, haemoglobin, and lactic dehydrogenase. Acetate, ketone bodies and especially free fatty acids were, he claimed, valuable indicators of the cows energy balance. While tests for all these substances could be automated, the analysis for free fatty acids would be slow and tedious and could not be built into a multi-channel analyzing unit. Lactic dehydrogenase was included by Kronfeld (1972) as an indicator of early cases of lymphosarcoma, a disease which does not appear to be present in the epidemic form in New Zealand (Rees, 1964; Shortridge and Cordes, 1971).

A further variation in the form of the profile has been reported from Sweden (Hewett, 1974). Even before Payne and coworkers had published their original paper (Payne *et al.*, 1970a) a series of tests had been drawn up for Swedish conditions which comprised haemoglobin, packed cell volume,

total leucocytes, serum calcium, inorganic phosphate , total protein, protein-bound iodine and iron. In a later stage of the project serum magnesium, urea nitrogen, sodium, potassium, albumin, globulin, total iodine, inorganic iodine and blood glucose were added. The profile now included all those anlyses which were present in the"Compton Profile". The iodine fractions were included because areas of Sweden were believed to be iodine deficient and leucocytes were included to detect the early stages of lymphosarcoma.

Other profiles are being studied, generally based on a similar range of parameters, but often including additional tests according to local needs (Stevens, 1975). Tests selected are usually based on those which are available for analysis through the Auto-analyzer SMA 12-60 systems.

In summary therefore, four major profiles have been suggested: the "Blowey Profile" restricted to keep costs down, and aimed at assessing protein and energy balance quickly and simply; the "Kronfeld Profile" used to assess energy balance and anticipate metabolic disorders as well as assisting in lymphosarcoma detection; the "Compton Profile" used to assess disease, nutritional status, management efficiency and to give some tag by which genetic superiority can be identified; and the "Hewett Profile" which is basically the Compton profile modified to suit local conditions.

Dougherty (1970) during his discussion of the original paper by Payne *et al.* (1970a) raised the following points in respect to the metabolic profile: -

(1) Metabolic profiles helped guide research by grouping abnormalities of calcium and magnesium metabolism with other metabolic disorders as part of a production disease complex.

(2) The state of herds and individual animals in relation to production disease might be assessed by using the metabolic profile technique.

(3) Some of the factors predisposing to disease in herds or individuals might be identified and even corrected with existing knowledge once identified by the metabolic profile.

(4) The metabolic profile might help in the selection of superior stock able to maintain homeostasis in spite of poor dietary intake and high production.

(5) It might be possible to predict potential future production from the metabolic profile of young animals.

A decade later the issues raised here have not been adequately answered; the whole field of the metabolic profile is, therefore, in need of further research and clarification.

B. SELECTED LITERATURE CONCERNING THE PARAMETERS STUDIED

Since the analyses described in the article by Payne *et al.* (1970a) are relatively simply carried out, and because most appeared relevant to the New Zealand situation, the approach adopted in this thesis has been to duplicate the Compton profile.

An extensive review of each of the 11 parameters examined was beyond the scope and needs of the investigation undertaken: instead a resumé of the literature related to the measurements used has been attempted particularly in respect to their homeostasis within the body, the values obtained by overseas workers, and factors that cause variation in these values.

Haematocrit (PCV)

In view of the comparative constancy of mean corpuscular haemoglobin and mean corpuscular haemoglobin content, there should be a direct correlation between haemoglobin level and PCV unless there is a clinical abnormality present causing an alteration in erythrocyte size. As a consequence of this relationship Payne *et al.* (1974) omitted the haematocrit from their later statistical summaries. Most of the comments concerning haemoglobin (covered in some detail in the next section) apply to the haematocrit and are not duplicated here.

A point which needs consideration when making haematocrit estimates relates to the anticoagulant used. Neither potassium nor sodium ethylene-diamine-tetra-acetate (EDTA) cause alterations in red cell size whereas sodium fluoride and potassium oxalate (F/O) (which is used to inhibit glycolysis as well as prevent coagulation) causes cellular distortion and alteration in the size of the erythrocyte (Medway and Prier, 1969). Heparin likewise seems satisfactory for haematocrit procedures since in a short trial on dairy cattle blood samples where EDTA, F/O and heparin were used as anticoagulants and compared, EDTA and heparin gave similar haematocrit values but there was a drop of almost 20% in PCV readings in the case of the F/O samples relative to the other anti-coagulants (Blackshaw, 1973).

The alterations in haematocrit by F/O anticoagulant has been compared between EDTA and a potassium oxalate - ammonium oxalate - ammonium fluoride combination (Manston *et al.*, 1974). While F/O caused the greatest degree of alteration, storage of the sample at $4^{o}C$ reduced the speed of the shrinkage of the red cells as well as the total shrinkage.

The concentration of the anticoagulant also influences the degree of erythrocyte distortion, an effect which can be reduced by ensuring that all tubes are filled to the maximum amount. This error can be reduced further by using larger collection tubes (7 ml) (Dubin *et al.*, 1976).

Another important source of variation in the haematocrit of cattle is associated with the technique of centrifuging (Schalm *et al.*, 1975). The small size of the bovine erythrocyte and the absence of rouleaux formation result in considerable trapping of plasma unless relative centrifugal force and duration of centrifuging are adequate. Fisher (1962) reported that 12,000G for ten minutes is required to pack bovine erythrocytes with a minimum of entrapped plasma.

Choice of anticoagulant and handling of the specimen at both the time of collection and during later centrifugation are therefore important points in technique to be kept in mind when determining the accuracy and repeatability of PCV estimates.

A range of values has been reported and the anticoagulant used must be considered. Schalm (1965) cited 33.6% ± 5.2 as a normal and it is unlikely in this case that the anti-coagulants used caused erythrocyte distortion. Values cited from work using F/O anticoagulant include 30.2% ± 2.8 (Payne *et al.*, 1973), 27.9% (Rowlands *et al.*, 1977a) and 30.0% ± 2.4 (Rowlands *et al.*, 1974b).

Haemoglobin

Haemoglobin is the respiratory pigment carried in the erythrocytes circulating in the blood stream. Since the cow is a comparatively lethargic animal, the level required is not as great as it is for dog, horse and man; nevertheless adequate oxygenation is essential for the tissues to carry out their function at a satisfactory level. Erythrocyte and haemoglobin synthesis are carried out at various locations throughout the body and the level of circulating haemoglobin is influenced by factors pertaining to the synthesis and duration of survival of haemoglobin pigment in the blood as well as changes in the fluid components of the blood. The average lifespan of the ruminant erythrocyte has been stated to be 47 days (Hansard *et al.*, 1959).

There are a number of factors to consider which have been reported to cause variation in haemoglobin levels in cattle: -

a) Season

Byers *et al.* (1952) in a survey covering a number of samples from cows kept both inside and outside recorded no difference in haemoglobin level that could be attributed to either season or to when the cattle were put out to pasture after being held in a barn over the winter period. Other reports record a rise in haemoglobin level in cattle at summer pasture (Kroncher *et al.*, 1927; Van Geller, 1928; Kroncher *et al.*, 1930; Rusoff *et al.*, 1954; Payne *et al.*, 1970b; Payne, 1972a; Rowlands *et al.*, 1974a; Hewett, 1974). Payne *et al.* (1970b) stated that during the winter indoor feeding period a decline in haemoglobin almost to the point of clinical anaemia appeared; this responded with a rapid rise in haemoglobin when the cattle were turned out to pasture in the spring. They suggested that this reflected the higher protein intakes on summer pasture as compared with those on winter feed (Payne, 1972a).

Whatever the seasonal effect on haemoglobin, the change in level, while having something of a cyclical nature,

does not return to the same point at the same time each year for each individual or even for the mean of the group (Payne *et al.*, 1967). Indeed the whole pattern of seasonal change needs to be examined under more controlled conditions as the reason for the development of an increasing percentage of anaemia in winter, the reason for the elevation of haemoglobin that occurs in the summer, and the effect of nutrition on haemoglobin levels all need clarification. There is also a suggestion (Page *et al.*, 1960; Abt *et al.*, 1966) that solar radiation has a direct effect on changes in the haemoglobin of both a seasonal and a diurnal nature.

b) Altitude and Climate

Clawson (1914) found a difference in erythrocyte numbers related to both altitude and climate. During summer there were increased erythrocyte numbers when the cattle had been moved to a higher altitude, whereas when the cattle were left at the same altitude erythrocyte numbers fell during the same period of time.

Manresa *et al.* (1934, 1939a, 1939b) in a series of papers recorded the effect of haemoglobin on native and imported cattle in the Phillipines. He reported a close reciprocal but negative relationship between haemoglobin indices and atmospheric temperature: these findings have been supported by the studies of Yousef and Johnson (1965), Bell *et al.* (1975) and Young (1975). The mechanisms by which this effect occurs have not been described but Schalm (1965) has suggested that water intake is a contributing factor, this being greatest during hot weather especially if the water is cooler than the environment. This excessive intake would expand blood volume and result in haemodilution.

c) Pregnancy and Parturition

Although the effects of pregnancy and parturition are considered slight the level of haemoglobin is higher during pregnancy than in early lactation (Lane and Campbell, 1969; Hewett, 1974; Rowlands *et al.*, 1975; Parker and Blowey, 1976; Treacher *et al.*, 1976). After cessation of lactation there appears to be a gradual increase in haemoglobin level (Hewett, 1974) until the increasing demands of a rapidly growing foetus bring about a reversal of this trend in the terminal stages of pregnancy (Morris, 1944; Hewett, 1974). Contrary effects have been reported with the rise continuing to parturition (McCay, 1931; Holman, 1956) and no effect of pregnancy at all has also been recorded (Conner *et al.*, 1967).

Parturition appears to produce some changes but the extent of these and their direction is not clear. The degree of variation in haematocrit increases from 4 weeks before to one week after parturition although around the actual event the level stabilises (Yoshida, 1974). A fall has been reported as occurring thirty six hours after parturition with haematocrit levels returning to normal over the next fourteen days (Morris, 1944). Most reports of change close to parturition appear to cover changes three days or more after parturition and are probably related to the initiation of lactation.

d) Lactation

McCay (1931) concluded that there was no relationship between the haemoglobin level and either milk production, fat production, or lactation length; other workers (Patterson *et al.*, 1960; Fisher, 1962; Lane and Campbell, 1969; Poulsen, 1974) have also recorded no significant alterations in haemoglobin level at peak lactation. More recently however Hewett (1974), Rowlands *et al.* (1975), Manston *et al.* (1975), and Treacher *et al.* (1976) have recorded a fall in haemoglobin level after the initiation

of lactation which persists generally for about three months and appears to be independent of nutrition. Rowlands *et al.* (1975) in their report noted a negative effect due to actual milk yield but this was small when compared with the effect of stage of lactation.

Payne *et al.* (1973) recorded mean haemoglobin concentrations of 12.7, 12.0, and 11.6 g/100ml for non-lactating, middle and high-yielding cows respectively. These differences varied from herd to herd, but were consistent throughout the year, with cows at peak lactation having mean haemoglobin concentrations 1.0 g/100ml and haematocrits 3.0% lower than non-lactating cows (Payne *et al.*, 1974). Rowlands *et al.* (1975), Kitchenham *et al.* (1975a) and Hewett (1974) all concluded that stage of lactation affected haemoglobin levels more than milk yield.

e) Nutrition

Greig and Boyne (1956) reported higher haemoglobin levels in monozygous twin calves fed on a high rather than a low plane of nutrition and suggested that the anaemia in the calves on the low plane of nutrition was a normochromic microcytic anaemia which would be consistent with protein deficiency in the presence of an adequate iron intake. This effect of diet was also recorded by Manston *et al.* (1975) who found marked differences between low and medium protein diets on both the haematocrit and haemoglobin levels in cattle particularly when they were producing high quantities of milk. The differences between diets were not significant before parturition or after lactation had ceased. Other workers (Hewett, 1974; Treacher *et al.*, 1976) have defined the time course of the changes more accurately and stated that, whatever the protein level in the diet, the haemoglobin and haematocrit levels fell during the first ten weeks of lactation and then began to increase in those animals fed high quantities of protein. Those on the low protein diet showed a similar increase only after they received high levels of protein at a later stage of the investigation.

It has been suggested by Little (1974) and Hewett (1974) that a fall in haemoglobin on a low protein diet may be due to a temporary slowing down of the synthesis of body protein in conjunction with the needs of milk synthesis of the udder. Such an effect has been shown for albumin synthesis (Little, 1974) in the liver at the beginning of lactation, but little is known yet concerning the effects of low protein diets on haemoglobin synthesis. Possible confirmation that this is the mechanism comes from the report of Payne *et al.* (1973) where they recorded that haemoglobin and albumin were correlated in one season and suggested that since albumin was an indicator of protein intake then haemoglobin was also sensitive to protein intake. However, as the rise in haemoglobin follows the rise in albumin after the correction of the protein level on a protein deficient diet (Smyth, 1976), haemoglobin is probably the less sensitive of the two as an indicator of protein sufficiency in the feed (Roberts *et al.*, 1978).

How sensitive haemoglobin levels are to protein intake remains open to question, however. Workers such as Manston *et al.* (1975) and Treacher *et al.* (1976) have noticed a lowering of haemoglobin levels when the protein intake falls below 16% (a figure McCay (1931) stated was adequate for haemoglobin synthesis), and Nomani and Evans (1972) recorded a rising haematocrit with a rising percentage of protein in the diet. On the other hand Parker and Blowey (1976) in their study of sixteen herds found little correlation between protein intake and haemoglobin level even though total protein intake in some of the herds was inadequate.

It is possible that nutritional factors other than protein are important in determining haemoglobin level. Energy for example may have some influence since cows secreting a higher fat percentage in the milk had a higher haemoglobin content in the blood than lower fat secretors (Byers, 1952); this could be a genetic effect. Iron,

which is essential for the formation of haemoglobin, is clearly another nutritional component and 70% of the iron contained in the body is in the form of haemoglobin (Church, 1971). Iron absorption from the intestine is generally low but the rate of absorption increases in iron deficient animals (Becker *et al.*, 1965). How the control is exerted is not known but it is thought to be related to both the rate of erythropoiesis and to the level of body iron stores. Pasture, especially clover, is rich in iron, and this leads to recovery from a developing anaemia when young animals fed on milk gain access to the pasture (Church, 1971).

f) Food Ingestion and Composition

The act of ingestion of food may have some effect on haemoglobin levels but this is not likely to be marked in a pasture feeding situation. When feeding of steers was *ad lib* and ingestion could occur at will no rise in haematocrit was recorded (Chase *et al.*, 1977b). However, when the food was placed before sheep on a once daily feeding regime Dooley and Williams (1975) recorded a decrease in the haematocrit measured from jugular blood just prior to their being fed. Once the feed was placed in front of them, and feeding commenced, an increase above the resting haematocrit was recorded. The increase started as soon as the sheep started to feed and was greatest thirty minutes later, an increase of 15% being recorded. Chase *et al.* (1977a) recorded the same findings in portal blood in steers fed on a twice daily feeding regime. This rise in haematocrit has been noted to be associated with a fall in extra-cellular fluid volume of 10-25%, an effect which was measureable fifteen minutes after the commencement of feeding and which reached a peak at 30 minutes after the start of feeding (Ternouth, 1968; Blair-West and Brook, 1969; Christopherson and Webster, 1972).

Food composition also appeared to have some influence on the response in that if the feed was dry, the elevation

was quicker and slightly larger (Ternouth, 1968; Christopherson and Webster, 1972; Dooley and Williams, 1976). It is probable that this is due to the increased salivary flow in animals on dry feed (Balch, 1958; Bailey, 1961).

g) Genetic Factors

An early report following investigation of monozygous twins stated that the haemoglobin content, red cell volume and red cell count showed little variation within the twin sets yet a large variation between sets (Anon., 1949); and Kay *et al.* (1976) similarly reported significantly less variation between identical twin calves than between the offspring from different dams for both haematocrit and haemoglobin levels. The concept of some form of genetic control is supported by the papers of Payne *et al.* (1970b; 1972b, 1974) who indicate that the major source of variation in the haemoglobin level is between herds rather than within herds and, since a closer within rather than between herd relationship is likely to exist in a genetic sense, it seems reasonable to assume that this is the underlying cause of this lower variation. Heritability estimates have now been calculated at 0.4 for haemoglobin (Rowlands *et al.*, 1974b) and 0.37 for haematocrit (Simon *et al.*, 1978). Some workers have examined the relationship between other factors which may have a genetic basis and the haemoglobin level. Payne *et al.* (1970b) examined the relationship between milk production and haemoglobin level and Schultze (1955), Arthaud *et al.* (1959) and Rowlands *et al.* (1974b) all examined the relationship between growth rate and haemoglobin. The correlations that existed lend further support to the existence of a genetic control for haemoglobin.

h) Age

Calves still on a whole milk diet show considerable variation which reflects the comparatively low iron content of milk (Church & Pond, 1974). Subsequent to

cattle becoming two years old this age effect was no longer observed (Wingfield and Tumbleson, 1973) although there is not complete agreement among investigators concerning this point. Heyns (1961b) and Hewett (1974) recorded that heifers had a higher haemoglobin level than mature cows and there are other reports of a decrease in haemoglobin with age (Shirley *et al.*, 1968; Lane and Campbell, 1969). However, although the age differences described were significant they were slight and fell within the mean $\pm$ 2 s.d. range accepted as comprising normal cattle in a metabolic profile (Payne *et al.*, 1970b). Hewett (1974) on the other hand carried out another trial on a large intensively farmed unit with zero grazing and an individual penning system; on this occasion heifers had low haemoglobin levels which rose until the fourth lactation and then remained constant. Other workers have recorded no change in haemoglobin with age once cattle had entered the milking herd (Byers *et al.*, 1952; Holman, 1956; Kitchenham and Rowlands, 1976).

i) Diurnal Rhythm

Unshelm (1968) carried out a series of experiments to test for the presence of diurnal rhythms in red cell numbers, haemoglobin and the haematocrit. Using permanent blood cannulae blood samples were collected from 12 cows every 2 hours between 0800 - 1800 hours over a three day period. Individual animal differences accounted for the greatest amount of variation observed. Significant day effects were found for haemoglobin and haematocrit although individual cows behaved differently on different days for all three parameters studied. Time of day variation was also significant for all three blood components with the values being highest in the morning and lowest in the afternoon - again significant interactions occurred between individual cows and time of day. The major shortcoming of the project was that feeding and milking remained at the same time each day. Both procedures could have influenced the results and they therefore can-

not be said to reflect an endogenous diurnal rhythm.

Abt *et al.* (1966) in their investigation recorded a plot for haemoglobin and haematocrit which showed a fall from a peak from 5.00 a.m. until 8.00 a.m., then a plateau, a further rise from midday with another peak at 3.00 p.m., a fall by 6.00 p.m. and a further plateau which was constant until 12.00 midnight. Their project suffered from the same shortcomings as Unshelm's (1968) project in that insufficient details were given in the paper to deduce whether the feeding and milking could have affected the haemoglobin and haematocrit values, and the collection did not continue for the whole of the 24 hours. The shapes of the curves for the diurnal changes however showed a marked similarity for both investigations.

It is possible that observed diurnal rhythms could be due to temperature changes. Manresa *et al.* (1934, 1939a, 1939b) for example found that haemoglobin levels followed a regular pattern, being highest in both morning and evening and lowest during late morning and early afternoon when the atmospheric temperatures were highest. These daily fluctuations were attributed to changes in the water balance with more water being consumed as the temperature rose. Brody (1949) confirmed that this was probably the case by reporting that although sweating was a relatively insignificant source of water loss in cattle, water intake rose with increasing ambient temperature, especially if the water was cooler than the atmospheric temperature. Provided cool water was available, blood concentrations tended to fall during a daily temperature rise; however this effect did not occur over a temperature range from 50 - 100^{o}F.

j) Breed

Reports in respect to breed differences in haemoglobin levels in cattle have also been conflicting. Anderson *et al.* (1930), Schalm (1965) and Ryan (1971) stated that Friesian cattle had the highest haemoglobin levels in the

dairy breeds; Byers *et al.* (1952) cited Jersey cattle as having a higher level of haemoglobin than Friesians; while Greatorex (1957) claimed the Jersey breed as having the lowest haemoglobin level among the breeds he tested including the Friesian. Kitchenham and Rowlands (1976) indicated that the haemoglobin level was higher in Friesian x Jersey cattle than either of the parent breeds; McCay (1931) found no statistically significant difference between breeds; and Mammerickx *et al.* (1978), in a survey covering a wide range of differing breeds, found that the only breed that showed a significant difference from the mean was the Charolais breed in Ireland. One factor which could have led to this conflict, is that in the vast majority of the projects described the different breeds came from different farms. Thus other influences such as nutrition, production and management, not described in the reports, could be accounting for the differences and not breed at all. Attempts to study groups of different breeds on the one property have resulted in groups too small to measure small differences with any degree of confidence. Should breed differences not result in significant variations from the mean, breed would no longer be an important consideration in the interpretation of the haemoglobin level in a metabolic profile. Regardless of breed, between-animal variation in haemoglobin level has been described as wide (Schalm 1965; Ryan, 1971).

k) Hydration of the animal and the physiological response to stress.

In interpreting the level of haemoglobin consideration must be given to the state of hydration of the animal. Bianca *et al.* (1965) investigated the effect of water restriction on the blood composition of steers and found that there was an increase in haematocrit with water deprivation; following the ingestion of water there was a transient rise before a return to pre-restriction levels. This transient rise was considered to be a result of splenic contraction in response to the excitement of having

access to water under conditions of extreme thirst. In a later experiment, when rehydration was performed through a rumen fistula, the transient rise did not occur (Bianca, 1970).

This problem of splenic contraction causing alteration to the haematocrit must be considered when collecting blood samples - results could vary with the temperament of the animal. Gartner *et al.* (1965; 1969) found an elevation in haemoglobin occurred as a result of the excitation of handling, but familiarisation with the handling procedure in the absence of pain associated with collection, caused a steady fall in the haemoglobin level so that after one weeks pre-conditioning the haematocrit and haemoglobin levels did not show a response. The circumstances of this trial, where a marked stress was applied, illustrated what could happen with quiet well-handled dairy cattle. A stress however, does not have to be extreme to induce a rise since Grenn *et al.* (1976) recorded an elevation in haematocrit after prolonged standing.

l) Clinical Disease

Finally, clinical disease can cause a decreased level of haemoglobin; for example parasitism (Ross and Todd, 1965) and scouring in calves (Garden and MacDonald, 1975) both result in lowered haemoglobin levels. Depression of quite a number of energy intensive body functions could result from lowered haemoglobin levels. One possible sequel to this is the reported increase in the number of services per conception that occurs with a lowered haematocrit (Rowlands *et al.*, 1977b).

Many of the sources of variation reviewed above will be cancelled out where numbers of individuals from a herd are sampled and the results pooled. Nevertheless there are measurable changes in both haemoglobin and haematocrit in lactating grazing dairy cattle which can point to inadequacies

in husbandry and management. The published evidence indicates that either of these two parameters is worthy of retention in the metabolic profile.

The normal value for haemoglobin in cattle has been reported as 11.0 g/100ml (Schalm, 1965); a number of other workers have cited differing values for the mean haemoglobin level, e.g. Payne *et al.* (1974) cite 12.0 g/100ml, while Parker and Blowey (1976) cite 10.2 g/100ml and Hewett (1974) cites 10.9 g/100ml. Between laboratory as well as between groups of animal differences could account for the variation in values observed.

Total Serum Protein and Albumin

Since albumin is one of the serum proteins and because changes in albumin level are likely to result in changes in the total protein level it is convenient to review total protein and albumin together.

Originally serum protein was thought to be a more or less homogeneous mass in the bloodstream. It was found however that chemical fractionation yielded two separate forms termed, because of solubility, albumin and globulin. Later (Tiselius, 1937) the globulin was divided by electrophoresis into alpha, beta and gamma fractions. However as the automated procedure used in the investigations reported in this thesis did not measure either the globulin or its different fractions they have been given little attention. It is appreciated that in future the smaller protein fractions could become of significance in profile development.

Proteins are hydrolyzed during digestion in monogastric animals to proteoses, peptones and peptides by the proteolytic enzymes secreted by the stomach. The only peculiar feature of the ruminant is that the source of the protein so digested generally comes largely from the rumen microflora. Further breakdown to amino acids occurs in the small intestine but complete breakdown to the component amino acids is not obligatory for absorption (Haurowitz, 1961). The amino acids and other protein breakdown products absorbed in the small intestine are transported to the liver via the portal circulatory system. Biosynthesis of new protein generally occurs in the liver although amino acids may be transported in the bloodstream for protein synthesis in other parts of the body, the most important of these being the gamma-globulins synthesized at various locations within the reticulo-endothelial system. The protein products in fact enter a dynamic pool with both plasma and tissue protein being called on to supply precursors for each other as the need arises (Dimopoullos, 1970).

Swick and Benevenga (1977) have put forward the concept of a labile protein pool based largely on the deposition in and removal of nitrogen from skeletal muscle protein. Under conditions of nitrogen deprivation the proteins of skeletal muscle supply amino acids for energy and for the synthesis of more vital proteins.

The production of plasma proteins differs from tissue proteins in a number of aspects. The plasma proteins must be able to be secreted by the cell in which they are synthesized into the plasma, the rate of synthesis being controlled, in a way not clearly understood, by the rate of breakdown in tissues remote from the secreting tissue. Furthermore there is a time lag before radioisotopes appear in plasma protein from labelled dietary amino acids (Green and Anker, 1955) whereas no such time lag occurs with tissue proteins (Anker, 1961). Even with the plasma proteins there are differences in turnover rate between albumin and globulin, the former being much higher (Jeffay and Winzler, 1958).

The problem is to define where catabolism occurs. There is evidence that it occurs in the liver and plasma (Haurowitz, 1961) and that some breakdown or loss occurs in the intestinal tract (Armstrong *et al.*, 1960; Armstrong and Tarver, 1960; Tarver *et al.*, 1961).

The serum proteins are involved in a wide variety of functions. As well as maintaining colloid osmotic pressure (Scatchard *et al.*, 1944), body pH, plasma viscosity and serving in the defence mechanism of the body, they combine with and act as the transport mechanism for a wide variety of endogenous and exogenous substances e.g. hormones (Antoniades *et al.*, 1957a, 1957b; Blumberg, 1960), haemoglobin, (Jayle *et al.*, 1952), metals (Martin and Perkins, 1953; Patras and Stone, 1961), bilirubin (Martin, 1949) and certain dyes. Their contribution to tissue protein synthesis and nitrogen balance, has already been noted.

Several factors may alter the level of serum proteins in the apparently healthy animal. Important among these are: -

a) Season

Total protein and globulin have been reported to fall late in winter (Ross and Halliday, 1976) after which their values varied considerably (Yoshida, 1974). At about the time globulin falls albumin starts to rise, it is high during the summer and low in early to mid winter (Payne *et al.*, 1972a; Payne *et al.*, 1974; Rowlands *et al.*, 1974a) though the change is not very marked. These movements in protein level with the season may be the direct effect of low temperature. In moderate cold ($8^{o}C$) the total protein was elevated but in acute cold ($-20^{o}C$) it fell (Halliday *et al.*, 1968) a factor believed to be due to a fall in globulin and a rise in albumin. Total protein has also been reported to be depressed at times of acute thermal stress (McDowell *et al.*, 1969).

b) Reproduction

The effects of pregnancy have been measured in a series of studies where it was found that prior to parturition a decrease in total protein of 10-30% was found (Larsen and Kendall, 1957; Larsen, 1958; Larsen and Hays , 1958). The data indicated that the immune beta 2 and gamma 1 globulins build up in the maternal blood for up to fourteen weeks before parturition and leave the blood when the colostrum is being formed in the mammary gland. On a quantitative basis all alterations were accounted for by the movement of the immune globulin components with the albumin remaining constant. These findings were confirmed by Dixon *et al.* (1961) who also reported that gamma-globulin was preferentially secreted into the cows udder during colostral secretion, resulting in a rise in serum total protein after drying off until the next colostral secretion, when it fell. Gardner *et al.* (1976) however reported that plasma protein values were only slightly altered around parturition while Rowlands *et al.*

(1975) found that the concentration of total protein and albumin both varied significantly with the stage of pregnancy or lactation. The most significant changes they recorded were in the last three months of pregnancy with the albumin level falling at or near calving and the globulin level rising. The interaction between dates of sampling and stages of pregnancy was particularly marked for albumin. Sykes and Field (1974) similarly demonstrated that albumin fell from early pregnancy to late pregnancy especially in the older animals, and in a subsequent report Sykes and Thompson (1978) showed a highly significant linear relationship between the change in albumin concentration during pregnancy or albumin concentration in late pregnancy and the calculated change in maternal body protein content. In their trials serum globulin was not affected by the protein status of the animal.

At the other end of the reproductive cycle there is a report by Rowlands *et al.* (1977b) that albumin has been found to be positively correlated to the number of services per conception.

c) Lactation

Generally albumin tends to be lower and the globulin level higher than normal in the lactating animal, although a number of workers have stated the difference is not significant (Heyns, 1961a; Payne *et al.*, 1973; Payne *et al.*, 1974); Rowlands *et al.* (1973) maintained there was a fall in total protein level associated with lactation; this has been reported as particularly noticeable during the first 30-35 days after parturition (Yoshida, 1974). Hewett (1974) found total protein to be elevated in the higher yielding cows. Apart from the emptying of immune globulins into the udder for the secretion of colostrum, the main changes in serum protein seem to be in the albumin with the globulin tending towards, though not always having, an inverse trend.

Little (1974) for example reported that albumin concentration was lowest in those cows which had recently calved and that there was a positive linear relationship between albumin and lactation for the first 120 days. The fall at calving was not necessarily consistent and arose, it was claimed, partly from decreased synthesis (mottled liver syndrome) and partly by loss from or dilution in the blood. Others have reported similarly (Sykes and Field, 1974). There appears to be a positive correlation between milk yield and albumin level (Kitchenham *et al.*, 1975a; Kitchenham and Rowlands, 1976). Other workers have found no differences in albumin concentrations among cows at differing stages of lactation or between lactating and non-lactating cows (Payne *et al.*, 1973; Payne *et al.*, 1974; Rowlands *et al.*, 1974; Rowlands *et al.*, 1975). Thus the correlation referred to above appears to occur only at peak lactation (Rowlands *et al.*, 1980a).

A relationship has also been described between an excessive fall in albumin in heifers entering the milking herd and the low solids non fat (SNF) content of the milk they produce (Payne *et al.*, 1974).

d) Nutrition

A number of workers have all reported that either a high or increasing protein intake results in a high or increasing serum total protein, particularly the albumin fraction (Nomani and Evans, 1972; Payne *et al.*, 1972b; Blowey *et al.*, 1973; Blowey, 1975 ; Manston *et al.*, 1975; Belyea *et al.*, 1975). This type of response was also cited by Payne *et al.* (1974) for cattle on summer pasture with a relatively high protein content where the haemoglobin, albumin and urea nitrogen concentrations all rose.

The plasma proteins are sensitive to various nutritional factors such as vitamins, growth factors and substances

which affect protein, lipid, and carbohydrate metabolism. A direct relationship has been found between Vitamin A and the albumin concentration of bovine serum (Erwin *et al.*, 1959). When Vitamin A was deficient the serum albumin fell but returned to normal ten days after intravenous carotene administration.

On a shorter term basis, temporary starvation did not produce any alteration in serum total protein but a small post-prandial rise was recorded (Coggins and Field, 1976). A deficiency of dietary protein on the other hand, as well as causing some depression in serum total protein, causes an alteration in milk production (van Horn *et al.*, 1976); twelve percent protein in the diet appeared to be critical in this respect.

e) Genetic Influences

Various reports of the influence of heredity on the plasma proteins of cattle have been published. Perk and Lobl (1959) found breed differences when comparing two markedly different breeds of cattle and were in fact able to postulate that the protein differences between the breeds had certain visible effects on the animal, e.g. disease resistance and thirst resistance. Kitchenham and Rowlands (1976) also reported a breed difference between Friesians, Ayrshires and their crosses and indicated that there were genetic influences on all serum protein fractions. A number of classifiable sub-types of beta globulin which are inherited have been recorded (Ashton, 1957; Smithies and Hickman, 1958) as have deficiencies of globulin, the most common of which, agammaglobulinaemia, has been characterised as a congenital defect (Perk and Lobl, 1962).

f) Age

Generally there is a decrease in the concentration of albumin and an increase in the globulin, principally gamma-globulin, as well as total serum protein with

advancing years up to about the age of five when the mean value tends to remain reasonably constant although individuals fluctuate (Garner, 1950; Dimopoullos, 1961; Little *et al.*, 1963; Schalm, 1970; Tumbleson *et al.*, 1973a; Kitchenham *et al.*, 1975a, 1976, 1977). In some cases it has been reported that the changes in albumin and globulin result in an almost constant total serum protein so that there is no change with age (Heyns, 1961b).

g) Diurnal Variation

Coggins and Field (1976) suggested that diurnal variation did exist but found that it was mainly related to feeding. In the same series of experiments discussed in the section on haemoglobin Unshelm (1969) studied the diurnal variation of the serum proteins. In his report neither protein nor albumin showed marked variation with the time of day.

h) Infections

The magnitude of the change that occurs in serum proteins during an infectious disease appears to depend greatly on the severity of the infection, the nature of the infective agent, and the inherent response of the host. This was illustrated by Jacox and Feldmahn (1956) who showed that experimental cases of pneumococcus infection in rabbits altered the protein profile less when the infection was treated with penicillin than when it was not. In the response to a bacterial infection there is generally an increase in the globulin concentration and a fall in albumin to keep colloid osmotic pressure within physiological limits. An exception to this appears to be in Johne's disease where virtually all protein fractions are lowered (Patterson *et al.*, 1968); however the overall nutrition of the animal must be considered with this disease.

With viral infections, on the other hand, until there are marked clinical signs of the disease there is little or no elevation of any fraction of the serum proteins even though there is antibody formation (Dimopoullos, 1961). This does not appear to be a universal finding since Perk and Lobl (1961) found an increase in gamma-globulin and a decrease in albumin in naturally infected cases of foot and mouth disease which they ascribed to alterations in hepatic function.

Of considerable importance under New Zealand conditions are helminth infections. Generally there is an unaltered total serum protein with a lowered albumin:globulin (A:G) ratio (Holmes and MacLean, 1971). In more extensive infections the decrease in albumin may be enough to depress the total protein level (Leland, 1961; Anderson *et al.*, 1965; Holmes and MacLean, 1971).

The relationship between globulin and disease has been mentioned; there also appears to be a relationship between albumin and disease as it has been reported in one trial that those calves which later developed a scour had a measureably lower serum albumin from birth than those which did not (Cabello and Michel, 1977). Garden and McDonald (1975) also reported a lower serum albumin associated with diarrhoea and felt that this was a dietary fault. They commented on the fact that low colostral intake from the cow resulted in a low gamma-globulin in the calf for the first few weeks of its life.

i) Hepatic Disorders

Since the liver plays a major role in the biosynthesis of the majority of plasma proteins, hepatic disorders are likely to be reflected in the plasma protein profile. In cattle an excessively high protein intake could result in breakdown of liver parenchymal cells and since calving and spring growth tend to coincide in this country the problem is more likely to occur in early

lactation rather than mid and late lactation or prepartum (Treacher and Collis, 1977). That this damage occurs has been demonstrated by the leakage of liver cellular enzymes to the plasma in early lactation.

j) Other Factors

Traumatic injury tends to result in a decrease in albumin and an increase in alpha-globulin for a few days. The effects of some other forms of 'stress' follow no clear pattern. For example Crookshank *et al.* (1976) reported that there was no change in serum total protein in response to either trucking, weaning or both and Healy and Falk (1974) obtained a similar result after subjecting sheep to rail transportation. Kriesten *et al.* (1976) found that the serum total protein was lower after trucking and sale while Gartner *et al.* (1969) found total serum protein to be elevated when cattle were in a state of excitation. In the context of 'stress' influences, dehydration could play a role since both albumin and total protein rise as water deprivation occurs and return to normal only two hours after watering (Wehmeyer, 1954). In the absence of dehydration no consistent effect on serum proteins has been reported as a result of high ambient temperature (Brody, 1949) and the only change that has been reported where short term food and water deprivation has occurred has been related to haemo-concentration (Healy and Falk, 1974).

There are considerable variations between different authors in respect to total serum protein and serum albumin levels for cattle. Examples include values of 6.97g/100ml and 3.2g/100ml (Bradish *et al.*, 1954), 8.08g/100ml and 3.37g/100ml (Decker *et al.*, 1959), 7.15g/100ml and 3.31g/100ml (Payne *et al.*, 1970b), 7.57g/100ml and 3.15g/100ml (Rowlands *et al.*, 1974), and 7.6 and 4.3g/100ml (Bogin *et al.*, 1974) for total serum protein and albumin respectively. Some differences could be accounted for by technique of measurement since the

total protein value of a blood sample rises simply on standing (Grenn *et al.*, 1976). However it seems more likely that the wide range of factors influencing serum proteins that have been reviewed would be responsible for the differences observed.

Despite the fact that albumin tends to show a wide variation about a group mean (Payne *et al.*, 1973) it is still considered a good indicator of the adequacy of protein intake (Smyth, 1976).

Urea Nitrogen

Protein digestion in the ruminant is basically a two step process with protein entering the rumen initially being degraded by microbial activity (el Shazly, 1952), chiefly the ciliated protozoa (Warner, 1956), and converted to ammonia which in turn is used extensively by the rumen microbes, especially rumen bacteria (Warner, 1956), for the synthesis of microbial protein. In fact ammonia rather than free amino acids appears to be the preferred substrate of a number of the rumen micro-organisms (Bryant and Robinson, 1962). However a quantitative analysis of rumen bacterial protein revealed that only 40% came from ammonia sources, the rest being absorbed from other nitrogen sources notably amino acids and poly-peptides even though these substances have a relatively short half life free in the rumen (Nolan *et al.*, 1976). There is considerable recycling within the rumen, i.e. when bacteria lyse, the protein is incorporated into further rumen bacteria. The microbial protein then passes on to the abomasum where it is subjected to enzymatic digestion as in monogastric animals.

In general the ammonia production rate is greater than the ammonia utilization rate (Tillman and Sidhu, 1969) and since blood urea concentration is related to rumen ammonia (Lewis, 1957; Egan, 1965; Weston and Hogan, 1968), factors affecting the rumen ammonia level are of great importance in interpreting plasma urea nitrogen concentrations. The level of digestible crude protein (DCP) is one of these and many reports attest to a close relationship, where the diet is relatively constant in other factors, between DCP and rumen ammonia levels (Preston *el al.*, 1965; Prewitt *et al.*, 1971; Muir *et al.*, 1972; Nomani and Evans, 1972; Payne, 1972b; Blowey *et al.*, 1973; Hewett, 1974; Blowey, 1975 ; Hewett *et al.*, 1975; Manston *et al.*, 1975; Parker and Blowey, 1976).

The nature of the protein is important with, for example, more soluble proteins leading to an increased amount of rumen ammonia (McDonald, 1952; Lewis, 1957; Tillman and Sidhu, 1969) and factors reducing protein solubility such as heat (Chalmers

et al., 1954; Waldo, 1968) or formaldehyde treatment(Ferguson *et al.*, 1967), by increasing resistance to microbial attack, result in a decreased rate of production of rumen ammonia. There are proteins of low solubility available to the animal which have little effect on rumen ammonia levels yet a high biological availability (Miller, 1979; Treacher, 1979; Treacher *et al.*, 1979). There is not complete agreement on the relationship between protein solubility and rumen ammonia levels however as Little *et al.* (1963) found no such relationship in their trial.

Various non-protein sources may also be used by ruminants to supply soluble nitrogen to the rumen microflora for conversion to microbial protein (Lewis, 1951). Urea is one such compound (Ekman, 1975). Higher levels of rumen ammonia and plasma urea nitrogen were produced from urea than with any other non-protein nitrogen supplement (Pal and Negi, 1977).

Another factor of major importance controlling the rumen ammonia level is the concurrent level of readily available energy. The synthesis of microbial protein from ammonia is energy dependant (Al Rabbat *et al.*, 1971) with increased energy intake facilitating protein synthesis and hence reducing rumen ammonia. This effect was demonstrated by Lewis (1957) who, by doubling the starch intake while maintaining protein intake constant, observed an immediate fall in rumen ammonia followed by a similar reduction in plasma urea nitrogen. Similar effects have been noted by a number of other workers (Mitchell *et al.*, 1940; Packett and Groves, 1965; Waldo, 1968; McIntyre and Williams, 1970 ; Parker and Blowey, 1976).

Only the uncharged ammonia molecule significantly diffuses from the rumen (Tillman and Sidhu, 1969) and ammonia thus absorbed passes to the liver where it is detoxified by conversion to urea. Unless critical levels of ammonia are reached in portal blood, all ammonia is converted to urea by the liver despite quite wide fluctuations in the uptake from the rumen (Blackburn, 1965; Tillman and Sidhu, 1969). If this criticial level is reached ammonia may pass into the peripheral

circulation and produce signs of toxicity (Lloyd, 1970). It is possible that some ammonia reaching the liver may be used for glutamic acid synthesis rather than conversion to urea (Cohen , 1974).

Not all ammonia entering the portal system does so by absorption through the ruminal mucosa and some ammonia not absorbed into microbial protein may spill over into the intestinal tract - this along with ammonia produced by further fermentation (particularly in the caecum) as the intestinal contents progress is able to be absorbed from further along the gastro-intestinal tract (Church, 1971). Furthermore ammonia absorbed from the rumen is not the only source of nitrogen for conversion to urea. Microbial protein, synthesized in the rumen, is digested in the small intestine and absorbed into the general body amino acid pool for the synthesis of body or milk protein - from this pool certain amino acids will be in excess of requirements. Some of these will be transaminated, others deaminated and the nitrogen moeity converted to urea. This may also be the fate of amino acids from degraded body protein. Enzymes necessary to the production of urea through the Krebs-Henseliet cycle have been shown to be present in the bovine liver (Joseph *et al.*, 1963). Overall production of urea is therefore dependant on the relative rates of flux through all of these pathways in the ruminant; the rumen ammonia level would however appear to be the most important.

The level of plasma urea nitrogen at any given time represents the balance between production, discussed above, and loss. Urea leaves the bloodstream either via renal excretion or through recycling to the alimentary tract. Only the former represents a loss of protein potential since urea returned to the rumen is hydrolyzed to ammonia and can subsequently be used for microbial protein synthesis. Of the urea returning to the rumen part is transferred via the saliva (McDonald, 1948; Somers, 1961; Cocimano and Leng, 1967) and part is transferred directly across the rumen wall (Houpt, 1959; Packett and Groves, 1965). Salivary urea concentration is determined largely,

though not entirely, by plasma urea levels (Somers, 1961). Total salivary flow may on the other hand be influenced by blood ammonia concentrations since high blood ammonia levels reduce flow (Oltjen *et al.*, 1969).

Transfer of urea across the rumen wall probably involves an active urease system in the epithelium (Houpt, 1959) and if urease activity is upset urea may be transferred across the rumen mucosa unchanged. Normally urea is transferred as ammonia which diffuses more readily (Houpt and Houpt, 1968) with the rate of transfer increasing with increasing plasma urea, until the serum urea level is approximately 45mg/100ml (Ford and Mulligan, 1970). Further increases result in only a slight increase in urea transport.

Despite detailed research, the exact mechanisms controlling renal urea excretion have still to be elucidated. Schmidt-Nielsen and co-workers (Schmidt-Nielsen *et al.*, 1957 1958; Schmidt-Nielsen and Osaki, 1958) found in the camel and sheep that urinary urea excretion was not linearly related to plasma urea levels but more to dietary protein intake. At low protein intake levels, a renal conservation mechanism comes into force and urea excretion becomes very low. The authors proposed a regulatory mechanism at the tubular level based on the active transport of urea from the ascending loops of Henle and augmented by a counter current multiplier system from the vasa recta. Their work has been successfully repeated (Clark, 1965) and the majority of workers have since shown that there is a relationship between plasma urea and urinary urea excretion which is non-linear at low plasma levels (or low protein intakes) due to this renal conservation mechanism (Cocimano and Leng, 1967; McIntyre and Williams, 1970; Ford and Mulligan, 1970; Thornton, 1970). The last named author reported that urinary urea clearance was related to both plasma urea level and urinary urea level and suggested that urine flow could be related to urea clearance. Verco (1969) however, reported that urea excretion was more closely related to urine flow than to either plasma urea levels or to dietary nitrogen intake.

Apart from input/output influences discussed in the preceding paragraphs there are other factors described in the literature which affect plasma urea nitrogen levels. These include: -

a) Seasonal Influences

Generally high levels of plasma urea nitrogen have been recorded during the summer months and low levels during the winter months (Payne, 1972b; Payne *et al.*, 1972a, 1974; Sykes and Field, 1974). This has been attributed to higher levels of protein in the feed when cattle are at pasture, possibly accompanied by a higher intake. On a true seasonal basis however, i.e. when diet remains constant, the opposite effect appears to occur as it has been reported that plasma urea nitrogen falls at the time of heat stress (Yousef and Johnson, 1965; Graham and Searle, 1966; Yousri *et al.*, 1977). This is apparently due to an increased excretion of urea in the urine (Graham and Searle, 1966) and is a function of heat adaptation since the non-native breed of sheep which suffered the heat the most (merino) showed the change in a more pronounced degree (Yousri *et al.*, 1977). Yet another effect due to season could be a reduction in water intake (Utley *et al.*, 1970; Little *et al.*, 1976) as it has been found that in cases of water deprivation the level of plasma urea nitrogen rises; this could be important if access to water was reduced at hot times of the year.

b) Lactation

Hewett (1974) recorded a steep rise in urea nitrogen levels during early lactation with a fairly consistent fall towards the end and suggested that this was probably associated with the amount of feed offered in response to the yield of the individual animals. Payne *et al.* (1973) however found that while individual plasma urea nitrogen levels showed a wide variation about a group mean, values were lower in the higher yielding cows, an effect that was re-affirmed in later work (Rowlands

et al., 1975). The most significant changes were confined to the three months on either side of calving with the plasma urea nitrogen levels at their lowest during the first month of lactation.

c) Genetic Influences

Breed differences have been recorded (Rowlands *et al.*, 1977a) and a high heritability derived through the analysis of daughter groups of sires that were examined (Kitchenham and Rowlands, 1976). The heritability has been calculated to be 0.49 (Simon *et al.*, 1978).

d) Age

Hewett (1974) reported a decrease in plasma urea level with age from the second to the fifth lactation but pointed out the differences were only slight and could have been due to chance. Kitchenham and Rowlands (1976) also reported that plasma urea nitrogen level decreased with age. Tumbleson *et al.* (1973b) on the other hand were unable to confirm any age effects.

e) Diurnal Rhythm

This has been reported (Coggins and Field, 1976) although it could have been due to the effects of feeding since it was found to take the form of a small post-prandial rise.

In summary it would appear that an increased concentration of urea in the plasma could be due to one or more of the following: -

(1) An increase in total protein intake.
(2) Feeding a more easily degraded protein, or a low level of non-protein nitrogen. This could lead to an increased production of ammonia in the rumen and subsequent urea production.
(3) Reduced energy intake (possibly accompanied by a fall in plasma glucose) leading to a reduction in microbial protein synthesis. There would probably be a concurrent increase in rumen pH and thus an increased uptake of

ammonia.

(4) Increased body tissue catabolism, as a result of illness and/or starvation; this could produce urea from de-aminated amino acids. What part this would play in increasing plasma urea levels is unclear.

(5) Extensive water deprivation, though this is unlikely in this country.

Low serum urea nitrogen values are going to be indicative of substandard feeding practices relative to the content and/or nature of the protein offered whereas high values may mean an excess of readily soluble protein with or without adequate energy. Provided the ammonia level in the portal blood does not exceed the liver's ability to convert ammonia to other products no major symptoms of toxicity should be observed.

There is however, one reported effect from increased nitrogen levels and this is on reproduction. Excess of nitrogenous fertiliser applied to the pasture has been reported to result in a fall in fertility (Stables and Bounds, 1969). Hewett (1974) also recorded a fall in fertility in two trials where high protein values and high urea nitrogen values were found respectively. As both could have been associated with a high protein intake he felt they were related. Other workers who have reported a negative correlation between protein intake and fertility include Girou and Brochart (1970) while Lotthammer (1974) has also stressed the dangers of feeding protein in excess of requirement following observations in German dairy herds where cows fed in this manner demonstrated a high incidence of post-parturient endometritis and anoestrus. He suggested that these symptons could be related to liver damage since herds where cows were fed excess protein had higher SGOT values than cows in other herds. The findings of Wettke and Jahn (1971), who reported that in 34 herds with infertility problems 56% of cows had abnormally high SGOT and bilirubin values, would support Lotthammer's observation since they attributed the abnormal values to either a low energy supply or excess dietary protein. The views advanced by the above authors are consistent with the theory of Sommer (1975)

who used SGOT and cholesterol levels to predict the incidence of periparturient disorders including mastitis and endometritis. Sommers however, did not suggest that these problems were due to an excessive intake of protein.

The actual levels of serum urea nitrogen recorded vary widely. For example Payne *et al.* (1973) gives a level of 14.4 ± 5.0 mg/100ml, Bogin *et al.* (1974) 30 ± 8.0mg/100ml, and Hewett (1974) 17.7 ± 10.2mg/100ml. If these are correct it would appear that the extremes of normality are wide and it is difficult to imagine an abnormal value. Two standard deviations below the mean in the case of Hewett's result would result in a negative value, something which is not possible and which suggests that serum urea nitrogen levels in the population sampled may not follow a normal distribution.

Glucose

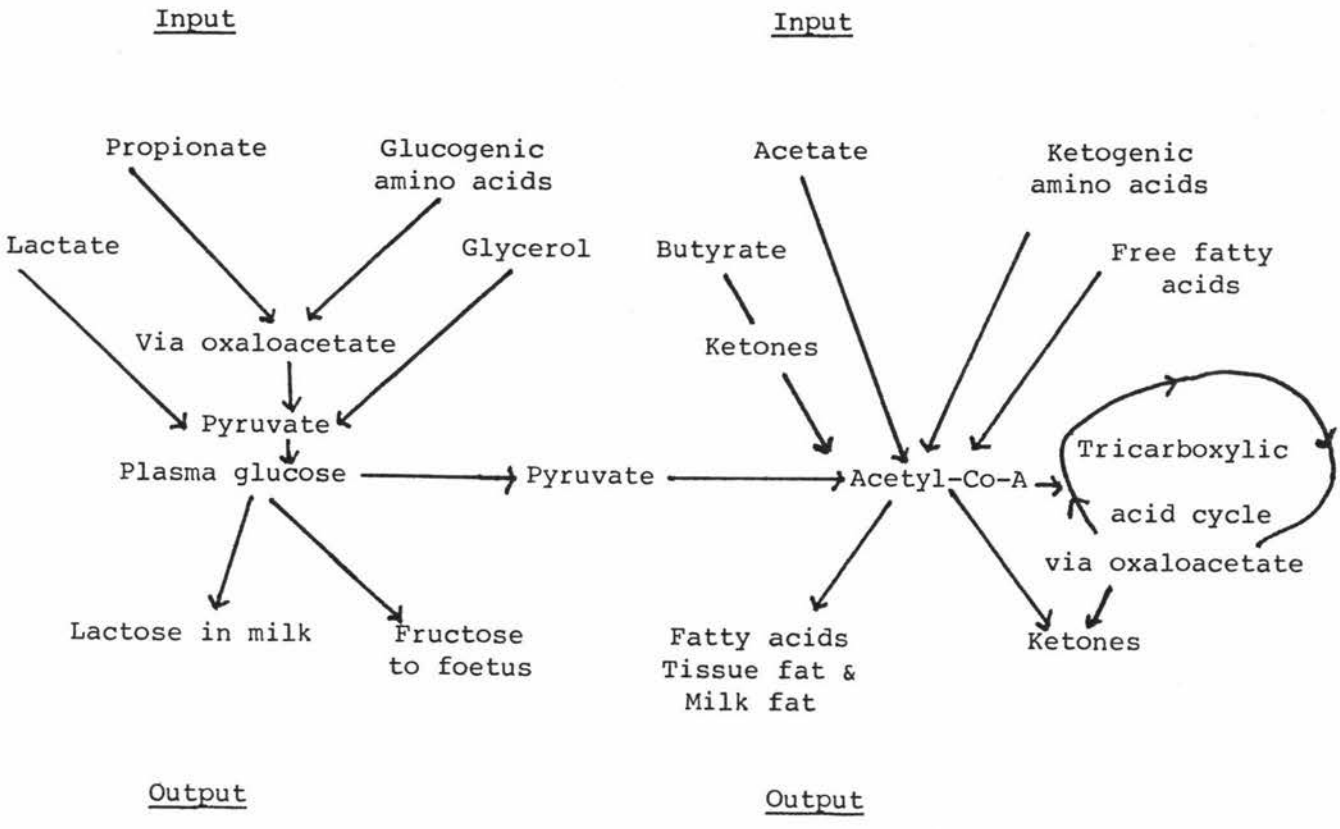
The process of rumen fermentation is normally so complete that until recently it was believed that almost no glucose was absorbed from the alimentary tract of the ruminant. However current research has shown that in cattle fed high levels of maize as much as 600g per day of starch may spill over into the abomasum and be subjected to enzymatic digestion in the same manner as in monogastrics (Wright *et al.*, 1966) resulting in the production of appreciable amounts of glucose. It has also been reported that when the percentage of starch in the diet is increased, the percentage of serum glucose obtained from propionate is reduced and therefore more is absorbed from the intestine as glucose (Judson *et al.*, 1968). Additional evidence for the direct absorption of carbohydrates is provided by the analysis of mesenteric vein blood for reducing sugars and comparing it with the carotid artery blood (Symonds and Baird, 1975). In each of two cows fed concentrates and hay the level of glucose was higher in the mesenteric vein blood than in carotid arterial blood and when one cow was fed ground maize and molassine meal the differential increased still further.

However propionate is a glucose precursor, and the other volatile fatty acids acetate and butyrate can be used as sources of metabolizable energy though not for gluconeogenesis. Propionate is therefore of key importance for glucose input. The relative amounts of propionate produced, as compared with the other volatile fatty acids, have been measured and found to vary with the type of diet. On diets composed mainly of roughage, the proportion of acetate predominates whereas on cereal diets, the proportion of propionate rises. Thus the intake of potential glucose-forming material depends not only on dietary energy intake, but also on the particular form in which the carobhydrate is provided (Blaxter, 1962).

The intermediary metabolism of carbohydrate and the pathways involved in the production of energy have been intensively investigated, especially in laboratory animals. Less work has been carried out directly on ruminants but enough is known to show that the processes involved are similar. Differences are chiefly quantitative and due to the peculiar nature of ruminant digestion and the unusual demand on glucose metabolism imposed by lactation and pregnancy. The major pathways in ruminant energy intake and output are shown in Fig. II:1 which has been prepared by Payne after consulting a major review on the subject (Krebs, 1966). Propionate and glucogenic amino acids predominate as potential glucose precursors while acetate is probably the chief non-glucose precursor providing inputs of energy via the rumen (Payne, 1970).

Kronfeld (1963) found that little glucose entered the peripheral circulation following a meal. This could be explained by a rise in circulating insulin in response to increased glucose absorption (Hart *et al.*, 1975). In monogastrics whole blood glucose level rises and falls according to proximity to a meal achieving a balance about six hours after the last meal; in ruminants it is only when glucose spillover to the abomasum occurs that this effect is possible. The amount involved is generally insufficient to affect circulating blood glucose. In the main, ruminant digestion produces volatile fatty acids and these, with other precursors produce glucose by gluconeogenesis. Bickerstaff *et al.* (1974) have gone so far as to claim that gluconeogenesis accounts for 98% of the glucose entry to the circulating glucose pool in the lactating dairy cow.

FIGURE II: 1. Pathways of energy metabolism in the bovine (from Payne, 1970).



Lactic acid is one of the acids produced by the breakdown of carbohydrates in the rumen, especially if the carbohydrates are present in a high concentration. This substance may be absorbed directly through the rumen mucosa and become a source of glucose via the lactic acid cycle. While most of the blood lactate is derived from the breakdown of muscle glycogen sodium lactate placed in the rumen causes an increase in both blood lactate and blood glucose (Hueter *et al.*, 1956).

Numerous studies have confirmed that acetate is not glucogenic (Kaneko, 1970) although acetate carbon does appear in body carbohydrate through the action of the tricarboxylic acid cycle. Propionic acid on the other hand is a well known glucose precursor and on a forage diet when little or no carbohydrate escapes rumen fermentation, propionate can

theoretically provide all the glucose not accounted for from amino acid precursors (Bergman *et al.*, 1966). One gram of propionate can theoretically yield 1.2g glucose (Kleiber *et al.*, 1953; Armstrong and Blaxter, 1957).

The role of butyric acid remains unclear. According to the beta-oxidation pathway it should produce acetate and be ketogenic. There is no known pathway which by-passes acetate and evidence from C^{14} labelled butyrate studies indicates that it enters this metabolic channel (Kleiber *et al.*, 1950). Butyrate however has been shown to have glucogenic properties in lactating cows (Kleiber *et al.*, 1954).

The remaining amount of glucose used by the cow has been calculated (Kleiber, 1959) as approximately 20% of the daily turnover and is the product of protein catabolism.

Glucose homeostasis is a result of the actions of several hormones which balance input and output of glucose from the circulating glucose pool in order to maintain a constant blood glucose level. As in monogastric animals, insulin is one of the key hormones in the control of blood glucose level, increased secretion of the hormone causing increased glucose utilization, increased protein and fat synthesis and at the same time decreasing gluconeogenesis and blood glucose level (West and Passey, 1967; Comline and Edwards, 1968). It has the direct effect of increasing permeability of the cell membrane to glucose as well as affecting those processes by which glucose is produced (Lindsay, 1970). This direct effect of insulin on glucose production has been confirmed by the direct infusion of insulin into the portal vein of sheep. Under these conditions the rate of glucose entry to the peripheral circulation was decreased (West and Passey, 1967). The depression in plasma glucose level is rapid and occurs in response to a glucose load; it has been assumed therefore that insulin is the feedback hormone correcting hyperglycaemia in normal healthy cows. As a consequence insulin is elevated after a meal and blood glucose

level does not rise markedly (Hart *et al*, 1975). Secretion of insulin is induced by a number of other metabolites besides glucose e.g. by butyrate and propionate in ruminants (Manns *et al.*, 1967; Manns and Boda, 1967; Horino *et al.*, 1968).

In monogastrics, the liver is able to absorb glucose as well as secrete it into the bloodstream. With ruminants however, the ability of the liver to take up glucose is reduced and this, coupled with the reduced sensitivity of the ruminant to insulin (Reid, 1951a, 1951b) means that changes in blood glucose levels to this hormone are not as marked as in the non-ruminant. The relatively low level of circulating blood glucose is also considered to be a function of the ruminant's reduced sensitivity to insulin.

Three other hormones that are important in the homeostasis of blood glucose are adrenaline, glucagon and the glucocorticoids. The first two hormones act on the enzyme systems in the liver which are responsible for glycogenolysis by increasing their level of activity (Rall *et al.*, 1956; Sutherland and Wosilait, 1956). Thus circulating adrenaline results in an elevation of serum glucose (Burtis *et al.*, 1966, 1968). The evidence for the involvement of glucagon in the glucose homeostatic mechanism is largely presumptive. Brockman and Johnson (1977) infused a glucagon inhibitor. This lowered the glucagon level, lowered glucose production and reduced the blood glucose level. Infusion of glucagon has also been found to bring about an elevation in serum glucose levels (Burtis *et al.*, 1968).

The glucocorticoids when administered parentally have a marked effect on circulating blood glucose levels although in one trial hydrocortisone did not significantly alter this level (Burtis *et al.*, 1966). ACTH has been found to keep blood glucose levels normal in starved cows (Saba *et al.*, 1966). The glucocorticoids stimulate gluconeogenesis and increase glucose production.

There may also be some relationship between growth hormone and glucose level. It has been found (Reynaert *et al.*, 1977) that ketotic cows have lower growth hormone levels, low glucose and high free fatty acid levels. Exogenous growth hormone does increase the glucose level by up to 10% (Williams *et al.*, 1963; Ventura and Head, 1968; Webb *et al.*, 1968). However, although there is this effect there does not appear to be a true feedback mechanism as falling blood glucose does not stimulate growth hormone secretion.

Thyroxine is yet another hormone which could have an effect on blood glucose levels as it has been found that exogenous thyroxine induced hyperglycaemia (Burtis *et al.*, 1966).

As with non-ruminants the liver appears to be the key organ in the stability of the glucose homeostatic mechanism, functioning either as a glucose releasing organ or as a glucose absorbing organ. The enzymes relating to gluconeogenesis are higher in bovine livers than they are in the livers of non-ruminants (Gallagher and Buttery, 1959).

Glucose still supplies approximately one third of the energy requirements of ruminants (Blaxter and Rook, 1955) and it has been calculated that the glucose requirement for a 500kg cow producing 25kg milk per day is 2,500g (Church, 1971). The brain is a glucose-using tissue (McClymont and Setchell, 1956; Gallagher and Buttery, 1959) and in ruminants the brain, like muscle, has a lower rate of utilization of glucose than is the case in monogastrics (Reid, 1950a, 1950b). Over half the glucose entering the cows bloodstream in a day is required to synthesize the cows milk (Kleiber, 1959) although this estimate is considered high by Annison *et al.* (1968). According to the last named authors glucose level is a limiting factor in milk production.

There are a number of short and long term factors which influence day to day glucose levels. These include: -

a) Lactation

There are fairly consistent reports of a rise in blood glucose level around parturition followed by a fall shortly after the animal has given birth (Godden and Allcroft, 1932; Merrill and Smith, 1954; van Soest and Blosser, 1954; Horrocks and Paterson, 1957; Oxenreider and Wagner, 1971; Payne *et al.*, 1972a; Kirchner *et al.*, 1977; Rowlands *et al.*, 1977b, 1980). It has been suggested that the rise in blood sugar is the result of increased secretion from the adrenal gland in response to the stress of calving (Merrill and Smith, 1954). There is also often a fall in serum calcium around calving (see later review on calcium) resulting in a decreased insulin secretion and/or release, an effect that is exaggerated in potential milk fever cows (Robertson *et al.*, 1960). Temporary cessation of feeding which may accompany calving could also result in the fall in blood sugar that some studies have reported at this time (Storry and Rook, 1962b; Kronfeld and Raggi, 1964; Radloff *et al.*, 1966; Baird *et al.*, 1972; Leat, 1974; Baetz, 1975). This fall is accompanied by a rise in non-esterified fatty acids (Economides *et al.*, 1973) which often bears an inverse relationship to glucose (West and Passey, 1967; Coggins and Field, 1976).

The fall in blood glucose which occurs in the early stages of lactation (Shaw, 1943; Leat, 1974; Hewett, 1974) is not easily explained. Rowlands *et al.* (1980) suggests that it occurs principally in high yielding cows which are unable to eat sufficient to meet their nutritional requirements. Treacher *et al.* (1976) however recorded a decrease in blood glucose level when the cows were receiving a ration calculated to supply total energy requirement. It has been suggested that it is the composition of the ration that matters rather than the energy content (McClure, 1977). Although early lactation does seem to cause lowered blood glucose levels there appears to be no correlation between either diet and blood glucose levels or between milk yield and blood

glucose levels.

b) Nutrition

Table II:1 illustrates the changes that occur in blood sugar in cattle that were fed once daily and trained to consume their feed in a relatively short time.

TABLE II:1: Changes in blood glucose in response to feeding (after Bines, 1968).

Time relative to feeding (minutes)	All hay diet mg/100 ml	All concentrate diet mg/100ml
-45	65.3	69.3
-15	67.1	69.7
30	60.8	63.1
70	55.1	58.4
120	53.3	47.5
170	56.1	44.1
225	60.4	47.8
288	62.8	50.3
345	62.3	65.1

A number of workers have recorded this fall in glucose after feeding (Simkins *et al.*, 1965a, b; Radloff *et al.*, 1966; Saba *et al.*, 1966; Payne *et al.*, 1972b; Coggins and Field, 1976; Hove and Halse, 1978). Others have emphasized the elevation of the blood glucose level following a slight fall after feeding (Thye *et al.*, 1970; Unshelm and Hagmeister, 1971; Jenny and Polan, 1975; Trenkle, 1978). Part of the fall in glucose levels after feeding may be associated with a loading effect by large quantities of volatile fatty acids. These volatile fatty acids depress non-esterified fatty acids and increase the insulin level thus resulting in a decrease in serum glucose (Church 1971). Another possible mechanism contributing to the fall in glucose could be through the production of acetate, particularly on a roughage diet, which is used mainly for fatty acid synthesis by extra-hepatic tissues (Cook and Miller,

1965). Acetate may contribute as much as half of the fatty acids of milk (Church, 1971) and their synthesis requires glucose to provide nicotinamine-adenine-dinucleotide-phosphate for the reduction reactions involved (McDonald, 1969).

Although the above reports suggest that time is important in relating blood glucose level to feeding this may depend on whether access to food is freely available or not. Chase *et al.* (1977a) for example showed that the glucose level in the portal blood of cattle fell after feeding on a twice daily feeding regime but when they were on a spontaneous self feed system this fall did not occur (Chase *et al.*, 1977b).

Quality and quantity of feed are also important determinants of blood glucose levels. At one extreme of the scale diets containing inadequate carbohydrate and an excess of protein can lead to ketosis and hypoglycaemia (Hibbit and Baird, 1967; Hibbit *et al.*, 1969). In less extreme circumstances results vary as it has been found in some trials (Manston *et al.*, 1977) that the blood glucose level tended to fall when the cattle were on pasture but remained high when they were on a grain diet whereas in others (Bartlett *et al.*, 1957) no alteration in blood glucose levels were observed when stall fed cattle were put on a grass diet. Seasonal differences which may affect feed quality, as well as quantity, would account for some of the variations observed since spring herbage has been found to be higher in soluble carbohydrate and lower in soluble nitrogen than autumn herbage (Ulyatt and Macrae, 1974). A number of workers have reported a rise in glucose levels during the colder periods of the year (Horrocks and Paterson, 1957; Halliday *et al.*, 1969; Mears and Groves, 1969; Payne *et al.*, 1972b; Hewett, 1974; Bell *et al.*, 1975). In extreme cold however ($-20^{o}C$), glucose after an initial rise was noted to fall (Halliday *et al.*, 1969), probably as it was used as

the energy source for shivering (Bell *et al.*, 1975). The rise recorded by Payne *et al.*, (1972b) was observed in the late winter period extending into early spring (January - March); they also noted lower glucose values in the summer months.

Blowey (1975) found that a decreasing or low blood glucose indicated a decreasing or low energy intake via the feed and vice versa. This in no way invalidated his earlier statement that almost all the significant changes in plasma glucose could be accounted for by changes in diet but not all changes in diet resulted in changes in the blood glucose level (Blowey *et al.*, 1973) since the actual relationship between the quantity of energy taken in and glucose level is not clear. McClure (1977) for example stated that metabolizable energy intake was related to blood glucose level in cows fed *ad lib* but not when they were fed on a restricted diet, i.e. where both total protein and energy were inadequate. If this is correct blood glucose levels may not be helpful in cattle managed on a grazing system since this frequently means restricted feeding. Nevertheless the occurrence of a low mean blood glucose level in a profile has generally been interpreted as an indication of a negative energy balance (Smyth, 1976).

c) Genetic Influences

In 1949 Brody stated that "blood glucose level is influenced by emotion, age, state of lactation, gestation and day to day, more than cow to cow" (Brody, 1949). His comment was based on the result of some work with identical twins, the inference being that environmental factors outweighed any genetic factors that may have been present in respect to the control of blood glucose levels.

This view has been questioned by Rowlands *et al.* (1973) who in a carefully controlled trial with growing calves estimated that genetic influences accounted for 74% of

the variation in blood glucose levels that were observed. Other workers who have recorded that blood glucose is under some form of genetic control include Wiener and Field (1971), Barber *et al.* (1975) and Rowlands *et al.* (1977a). The latter report referred to breed differences between bulls where animals of the Sussex breed were found to have higher blood glucose levels than those of the Lincoln Red which again were higher than those of the Devon breed. Kay *et al.* (1976) however reported that glucose levels were no more stable within identical twin pairs than between single animals in the same herd.

d) Age

The calf tends to behave as a monogastric and have high blood glucose levels compared with levels observed as it matures (Little *et al.*, 1977). Other workers have found that once the animal has matured, there is a fall in blood glucose from first lactation to the third lactation (Hewett, 1974; Henricson *et al.*, 1977) and then it rises irregularly from there (Hewett, 1974) possibly associated with level of milk production.

e) Excitement and Stress

The effects of 'emotion' mentioned by Brody (1949) have been interpreted as an increase in circulating adrenalin brought on by the apprehension of the sampling procedure causing an elevation of blood glucose. Other 'stressful' states may cause alterations in glucose levels depending on the individual animals reaction to the stimulus and to any feed deprivation that may result from it. Thus Crookshank *et al.* (1976) reported no change due to weaning, trucking or both, yet other workers (Kriesten *et al.*, 1976) found that glucose was elevated after trucking. Excitement and oestrous behaviour were found to elevate blood glucose level (Hewitt, 1930; Hodgson *et al.*, 1932) while high ambient temperature has been found to result in depression (Riek and Lee, 1948; Brody, 1949).

Just as volatile fatty acid infusions depress non-esterified fatty acid (NEFA) levels, an energy deficit brings about increased mobilization and testing the level of non-esterified fatty acids has been used as a measure of energy deficit. Although NEFA have been reported as a more sensitive indicator of the nutritional status of a lactating cow than blood glucose, the very variable nature of the relationship in early lactation limits its usefulness (Erfle *et al.*, 1974). In an earlier investigation NEFA and ketones were found to be a good measure of nutritional status in sheep but not cattle (Bowden, 1971).

High NEFA components in the blood stimulates milk fat secretion (McDonald, 1969) and in the presence of inadequate energy intake there is a fall in the proportion of fat : solids non-fat in the milk. This change has also been suggested as a measure of energy intake (King, 1968). Flux and Patchell (1954, 1957) had also found the balance of fat and solids non-fat in the milk to be a relatively sensitive indicator of dietary inadequacies and Foot *et al.* (1963) recorded a carryover effect on milk composition when heifers had an inadequate nutrition pre-partum. Munford *et al.* (1964) in their trials induced under-nutrition by depriving cattle of all pasture between two consecutive milkings - milk yield was reduced and an elevation in milk fat and a fall in solids non-fat resulted. While solids not-fat and milk fat percentage do alter in relation to a variety of influences, including age and stage of lactation, these alterations tend to be less than the alteration in yield, and what is more important in the context of this review, less than the alteration due to nutritional influences (Sargent *et al.*, 1966). Nevertheless it has been claimed that milk components do not bear a good relationship to energy status in the early lactation period (Payne *et al.*, 1973).

There are three important effects of hypoglycaemia. The first of these is on lactation - the mammary gland appears to have a priority for glucose (Bartley and Black, 1966) and milk secretion is directly related to a continuing adequate

supply of glucose to the mammary gland (Kronfeld, 1972); when the glucose level in the perfusate of an isolated udder was reduced below 30mg/100ml secretion stopped (Hardwick *et al.*, 1963).

The second effect is the positive correlation between glucose level and growth rate recorded by a number of workers (Payne *et al.*, 1970b; Rowlands *et al.*, 1974a; Kitchenham *et al.*, 1975b). There is not unanimous agreement on this relationship however as indicated by the papers of Schultze (1955) and Arthaud *et al.* (1959). Little *et al.* (1977) have since clarified the matter by demonstrating that the blood glucose levels were in essence measuring feed intake and, at higher feed intakes, weight gains were faster.

The third effect is the relationship between reproductive performance and blood glucose level. A number of trials have been carried out in an attempt to elucidate the relationship between hypoglycaemia and reproductive failure (McClure, 1968); hypoglycaemia was often artificially induced and frequently accompanied by a very marked weight loss; blood glucose estimations were really not required to identify this problem. With the advent of the profile however, a further look has been taken at this relationship. A better understanding of the report by King (1968), that cows gaining weight had higher conception rates than those losing weight, was achieved in a trial by Downie and Gelman (1976). They found that lowering dietary energy to 0.95 x maintenance requirements resulted in a fall in body weight which continued for the ten week trial period. There was an initial fall in blood glucose followed, after four weeks, by a rise, and at the end of the ten week trial period glucose levels had almost reached the levels that were present before food restriction began. When body weight and glucose level were falling, fertility was depressed, but when body weight was falling and glucose level was rising, the fertility was satisfactory. Other trials have shown that those cows which hold to first service have significantly higher blood glucose levels than those that do not (Parker and Blowey, 1976;

Hunter, 1977). The difference, while significant, is small and of little practical importance.

Further studies of profile results in herds that were hypoglycaemic noted a relationship between first service non-return rate and hypoglycaemia (McClure and Payne, 1978) and this was related to the degree of hypoglycaemia. When animals were not hypoglycaemic no relationship between blood glucose level and first service non-return rate existed. Garden and McDonald (1975), following profile investigations, were able to correct poor reproductive performance in two herds that were found to be adversely affected by hypoglycaemia in north-eastern Scotland when they implemented dietary changes.

Hewett (1974) on the other hand recorded no relationship between glucose and reproductive performance in his series of profile studies. Hyperglycaemia does not normally occur except as part of a disease picture (Phillips *et al.*, 1971).

The glucose levels recorded in the U.K. have been measured on whole blood. Since there is little interchange between glucose in the erythrocytes and plasma glucose in the bovine, a correction factor based on the haematocrit can be applied to change whole blood glucose values to plasma glucose values (Whittard and Rose, 1971). Two values which have been given are 45.4 mg/100ml for whole blood (converting to 54.0 mg/100ml for plasma - Payne *et al.*, 1973) and 52.0 ± 8.0 mg/100ml for plasma for cattle in Israel (Bogin *et al.*, 1974).

Sodium

Sodium is an ubiquitous element, present in all body tissues especially in the blood plasma and interstitial fluids. Apparently sodium makes a greater contribution to pH buffering systems and the maintenance of a physically desirable osmotic pressure than does any other inorganic nutrient and it is involved in the regulation of extracellular fluid volume. The transmission of nerve impulses is related to the electrical potential resulting from the separation of sodium and potassium across the cell wall since the nerve action potential is associated initially with the inflow of sodium which precedes a sudden outflow of potassium (Church, 1971).

Intake of sodium is largely through feedstuffs; water containing sodium may be consumed but usually only in small quantities since salt water is unpalatable (Denton, 1965). The level of intake recommended is 10g/day of salt for young beef animals and 14-21g/day for lactating animals (Anon, 1965). Smith and Aines (1959) however estimated the salt requirements for an adult cow producing 20kg milk per day at 30g salt per day indicating that the above requirement was not sufficient.

Sodium has a differing degree of digestibility at a constant dietary level in different types of feeds. The sodium in white clover for example was found to be 85.4% available while the sodium content of perennial ryegrass was 61.1% available (Joyce and Rattray, 1970a). Although this trial was conducted on sheep the digestibility of sodium in cattle is probably similar. In feedstuffs therefore the availability of sodium would appear to be neither total nor constant and faecal losses are positively correlated to both dry matter intake and total sodium intake (Elam and Autry, 1961; Renkama *et al.*, 1962a, b). Other workers however (Payne *et al.*, 1972b) report the digestibility of sodium as being virtually 100%.

Kemp (1964) found that certain pasture species contained inadequate sodium for minimum requirements of lactating cattle and that the variation of sodium level within a pasture species could be considerable. Payne *et al.* (1972b) also reported a considerable variation in levels of sodium in pasture with a low of 0.14%; sodium deficiency could develop under these circumstances particularly if the dietary sodium was of low availability (Joyce and Rattray, 1970a). A fall in serum sodium in grazing dairy cattle, particularly those at peak lactation, has been recorded in the summer (Payne *et al.*, 1974) indicating that at this time of year pasture may contain inadequate sodium to meet the demand.

Part of this hyponatraemia could be explained by the relationship between sodium and potassium since in cases of a low sodium intake, an increase in the potassium in the feed increases the irreducible losses of sodium in the faeces (Church, 1971). Other workers (Dobson *et al.*, 1966) have similarly found that sodium excretion altered according to diet and have postulated that differing water intakes with the different diets could be responsible. This would affect the sodium loss irrespective of the potassium content of the feed, because of losses in the increased urine flow that would result.

The seeking of salt by the ruminant is an important aspect of sodium regulation, a mechanism which is utilised practically in the provision of salt licks for animals. In the wild state the only animals which have been observed to take the licks are the herbivores (Tasker, 1970). It has been suggested that the salt appetite of ruminants is related to sodium and potassium metabolism by a mechanism which monitors the active transport of the two ions at a given location (Michell, 1974). An important and so far unexplained factor in the seeking of salt and the replenishment of body reserves is that sheep, when offered a salt containing solution, were able to stop drinking when enough sodium to replenish body stores had been consumed but long before it would have been possible for the

sodium to have been absorbed to restore the plasma balance (Denton and Sabine, 1961, 1963).

Licking is one of the signs of a sodium deficiency (Smith and Aines, 1959; Whitlock *et al.*, 1975) though licking and pica have not been reported by others (McClymont *et al.*, 1957; Devlin and Roberts, 1963; Hawkins *et al.*, 1965; Kerk, 1968). Smyth has gone as far as to suggest that licking may be simply a habit that cattle adopt (Smyth, 1976). Denton and Sabine (1961; 1963), while not reporting pica or licking, did report a salt craving manifest by drinking the urine of other cattle especially if they had been sodium supplemented. It is possible that hypotonicity of the blood reacts via a hypothalamic centre and promotes this salt seeking. While it has been proposed that sodium appetite may be regulated by a central control mechanism similar to that for thirst and appetite (Andersson and Olsson, 1970), oral and ruminal receptors have not been located, nor have brain receptors to aldosterone or angiotensin. Appetite level is however directly related to the degree of sodium deficiency.

An increase in the sodium content of the blood may occur in either water deprivation (Little *et al.*, 1976) or following artificial supplementation. In a series of experiments Weeth and coworkers (Weeth *et al.*, 1960, 1961, 1962, 1968) studied the effect of excess salt consumption by cattle in various situations. They found that cattle were able to tolerate a higher salt load in the winter than they were in the summer. When the animals started to suffer ill effects serum potassium was observed to be elevated as well as the serum sodium and there was also an elevation of haematocrit.

The ability to tolerate salt in the drinking water varies from individual to individual and sheep drink sodium-containing water in differing amounts depending on their haemoglobin type (Michell, 1975a). Such a genetic link could be used to identify animals which would be more successful in some environments.

In cases of water deprivation, or when there is an excessive intake, both sodium concentration and the osmolarity of the serum rise to a plateau (Little *et al.*, 1976). A rise in osmotic pressure of 1-2% is one factor leading to the development of thirst (Holmes and Gregersen, 1950).

While the bone acts as a small reservoir of sodium (approximately equal to a lactating cows daily requirement) which may be called upon in times of a negative sodium balance, there is evidence that all the sodium in the body, including that in the bone and the gut, is in a fairly rapid state of exchange (Payne *et al.*, 1972b). Sodium is lost from the body by a variety of mechanisms of which sweating is one. The rate of sweating in the bovine is relatively low though it does increase slightly as atmospheric temperature rises (Payne *et al.*, 1972b).

Sodium is also present in the various secretions of the intestinal tract, particularly saliva where in cattle sodium content is especially important because of the large amounts that are secreted (Denton, 1957). The main component of this is the alkaline parotid secretion of 50-150 litres per day (Bailey and Balch, 1961). In a marginal deficiency the proportion of sodium in the saliva changes from 180 : 5 sodium : potassium to 20 : 160 (Denton, 1957) with the extent of the change being proportional to the degree of sodium deficiency. It has been suggested that this is a sodium conservation mechanism as the amount of sodium passed in the saliva is reduced yet the osmotic pressure, essential for the maintenance of rumen osmotic pressure for normal digestion to proceed, is held constant (Kay and Pfeffer, 1970). Evidence that sodium conservation is the reason for this mechanism is provided by the fact that at higher flow rates of saliva, its ionic composition approaches that of serum (Blair-West *et al.*, 1970) and exogenous aldosterone, as well as reducing urinary loss, also reduces the concentration of salivary sodium (Blair-West *et al.*, 1963a).

The results of this mechanism are beneficial in two ways. Since sodium may be replaced by potassium in the saliva while sodium is still being absorbed from the rumen, the rumen is capable of acting as a buffer in times of a short term sodium deficiency (Blair-West *et al.*, 1963a, 1965; Murphy and Gartner, 1974). Furthermore it has been suggested that the sodium : potassium ratio may be an excellent measure of the sodium status (Kemp and Geurink, 1966; Schellner *et al.*, 1971) and a method for the field collection of saliva has been described (Murphy and Connell, 1970).

Most of the sodium absorption that occurs apparently takes place in the lower segment of the small intestine (Smith, 1962; Mylrea, 1966; Perry *et al.*, 1967; van T'Klooster, 1969; Grace *et al.*, 1974) although some, especially if the contents are hypertonic (Parthasarathy & Phillipson, 1953) takes place from the rumen. There is a secretion of sodium into the abomasum and upper segments of the small intestine (Smith, 1962; Mylrea, 1966; Perry *et al.*, 1967; Grace *et al.*, 1974).

It is possible for large quantities of sodium to be lost in the abundant watery faeces of the dairy cow even though the level of sodium in them is hypotonic relative to that in the serum (Goodall and Kay, 1968). Despite Brouwer's report that faecal sodium remained relatively constant at 18mEq/l (Brouwer, 1961) many other workers (Renkema *et al.*, 1962b; Blair-West *et al.*, 1965; Helfferich *et al.*, 1966; Bertzbach *et al.*, 1966; Pfeffer *et al.*, 1970) have recorded a fall in faecal sodium where sodium intake was reduced and Elam and Autry (1961) have indicated that the reverse occurred when sodium intake was increased.

The most important route of sodium excretion, and the one by which balance is effected, is through the kidney. More than 90% of the sodium filtered off through the glomeruli is resorbed in the proximal tubules and the loops of Henle regardless of the sodium status of the body. The balance of

the sodium remaining in the filtrate may also be absorbed or it may be excreted in the urine depending on the needs of the body (Church, 1971).

The hormone aldosterone is intimately involved in this regulatory process and it has been shown (Sonnenblick *et al.*, 1961) that during a sustained water diuresis, exogenous aldosterone caused not only a reduction in the sodium concentration of the urine but also, after a sixty minute latent period, substituted potassium in place of sodium. Denton (1965) however, has questioned the role of aldosterone in the sodium homeostatic mechanism as at circulating physiological levels (compared with single doses) there does not appear to be a response by the kidney, while Blair-West *et al.* (1968) have suggested from their experiments, that aldosterone may be of importance in sodium conservation only during a negative sodium balance and that it may not be of significance in minor day to day adjustments.

It is apparent that the control of aldosterone is poorly understood. For example it seems that exogenous ACTH stimulated secretion of aldosterone but not when given in the physiological range (Blair-West *et al.*,1963b) while in sodium deficiency aldosterone secretion was elevated but cortisol - cortisone which should give an indication of elevated ACTH, was not increased. There is evidence however, that in sodium deficiency angiotensin secretion is stimulated and that this is the factor that stimulates aldosterone since angiotensin appears in the circulation before aldosterone (Barger *et al.*, 1958; Blair-West *et al.*, 1968). The effect of aldosterone appears to be on the tubules so that in sodium retention water is not simultaneously retained; this is consistent with the time lag of 20-60 minutes in the effect of aldosterone that is observed (Sonnenblick *et al.*, 1961).

Other hormones and/or mechanisms may be involved as well as aldosterone as it has been found that sodium was excreted in greater amounts by the kidney during exogenous administration

of both parathyroid hormone (Bijovet *et al.*, 1972), and calcitonin (Bijovet *et al*, 1972) and hypertonic saline infused into the third ventricle in the goat similarly provoked a natriuresis (Andersson *et al.*, 1967, 1969). In all these reports 'pharmacological doses' have been given and the responses that would occur at physiological levels remain unknown.

Other factors that have been associated with serum sodium levels include: -

a) Season

Seasonal effects have been reported (Payne *et al.*, 1974; Hewett, 1974) but these have been related to differences in the sodium content of the feed (Rowlands *et al.*, 1974). Temperature, in the presence of an adequate sodium intake has no effect on serum sodium levels (Yousef and Johnson, 1965).

b) Reproduction Cycle

Rowlands *et al.* (1975) reported that serum sodium tended to increase in the non-lactating cow in the later stages of pregnancy and then fall after calving; this is consistent with an earlier finding that aldosterone secretion is elevated in the third trimester of pregnancy to meet the increasing demands of the foetus (Watanabe *et al.*, 1963). Housed sheep have shown a preference for sodium in the luteal phase of successive cycles compared with the oestral and post-oestrous stages (Michell, 1975b). The increase in sodium appetite followed increasing progesterone levels. Cattle have not been examined to determine whether they behave in the same way. Hypo-natraemia has been reported to be associated with a higher than normal incidence of cystic ovaries and irregular oestrous periods in cattle . A causal relationship has however not been established.

c) Lactation

Under normal circumstances it has been suggested that the extra sodium required for milk secretion is obtained by a larger food intake and increased urinary retention of this electrolyte (Lomba *et al.*, 1969b). Unless dietary sodium was inadequate lactation should therefore have little effect on sodium levels. Subclinical and clinical mastitis may alter this balance since the sodium content of milk rises by at least 100% when these conditions are present (Linzell and Peaker, 1972). Thus in the case of an animal struggling to maintain sodium homeostasis it seems feasible that an attack of mastitis could be the triggering factor leading to the development of hyponatraemia.

d) Genetic Influences

Healy and Falk (1974) found no difference due to breed but Rowlands *et al.* (1977a) did record significant breed differences, the Sussex breed being higher in serum sodium than the Devon which in turn was higher than the Lincoln Red.

e) Age

A number of workers have reported no change in serum sodium with advancing age (Tumbleson, 1973 ; Bide *et al.*, 1973; Healy and Falk, 1974) whereas Kitchenham *et al.* (1975a) reported that serum sodium tended to decrease with age. Because of the importance of this electrolyte in osmotic regulation changes with age are not expected.

f) Diurnal Influences

It has been found that sodium decreases during the day till noon and then increases again to show a maximum at 1600 hours. This is a reciprocal pattern to potassium and could be the result of diurnal changes in the adreno-cortical hormones (Unshelm and Hagmeister, 1971).

g) Stress

Stress may effect the level of serum sodium possibly depending on the level of sodium repletion of the animal prior to the event as Kriesten *et al.* (1976) reported a lower serum level following trucking while Crookshank *et al.* (1976) reported no effect due to trucking. Neither did the 'stress' of acute starvation of pregnant sheep produce any alteration in serum sodium level (Wolff *et al.*, 1974).

h) Sex

Healy and Falk (1974) found little difference due to sex but Doornenbal (1977) found that females had higher serum sodium levels than males. It appears that the issue is unresolved.

Signs of sodium deficiency reported in various articles include dullness, listlessness, inappetance, polydipsia, polyuria, salt hunger, pica, weight loss and ultimately total cessation of milk production. Terminally there is muscle tremor, staggering, irregular heart beat, tetany and death (Smith and Aines, 1959; Hawkins *et al.*, 1965; Whitlock *et al.*, 1975). The plasma level has been monitored and fell from a normal of 135mEq/1 to 131mEq/1 (Whitlock *et al.*, 1975): thus relatively small changes in the plasma level may be significant.

The actual level of sodium quoted in the literature varies quite markedly. Dale *et al.* (1954) obtained 162mEq/1 using plasma and Reihart (1939) a level of 155mEq/1 with serum. Lower levels have been reported and 138-144mEq/1 is more common (Sellers and Roepke, 1951 ; McSherry and Grinyer, 1954; Evans and Phillipson, 1957; Spector, 1958; Bogin *et al.*, 1974; Payne *et al.*, 1974).

Potassium

Potassium is the principal intracellular cation and is high in cells and low in the extracellular fluid; in humans for example only 1.75% of total potassium is found in the extracellular fluid (White *et al.*, 1964). Despite the fact that potassium concentrations in the extracellular fluid are low they are maintained within relatively narrow limits (3 to 7 mEq/l) and variations may cause severe changes in cardiac muscle action even leading to death (Bergman and Sellers, 1954). Since there is a concentration gradient for both sodium and potassium across the cell wall it was initially thought that there was an active force maintaining this gradient referred to as the sodium pump, and that diffusion of potassium into the cell was a passive osmotic process. Subsequent evidence however has shown that there is a separate active process pumping potassium into the cell also (Stevenson and Wilson, 1963).

Potassium along with sodium is concerned with nerve irritability and transmission of nerve impulses. Hence nerve fibres are rich in potassium, and when a nerve is stimulated potassium rapidly diffuses out of the cell and is replenished during a rest period (Church, 1971).

Intracellular potassium plays a critical role in the actinomycin-adenosine triphosphate system of muscle and contraction of muscle releases potassium to the extracellular medium part of which is replaced in the muscle cell by sodium (Church, 1971). This results in a variation in potassium content between the various tissues and in cattle, muscle contains more potassium than any other tissue, although bone and gastro-intestinal tract and contents are also relatively high.

A tissue deficiency of potassium and sodium results in lethargy while an excess results in hyperirritability, effects which are virtually the reverse of the situation with calcium and magnesium.

Like sodium, potassium is ionised and appears to be in constant transfer within the body; it is easily measured and balance studies simply require evaluations of the input and output. Intake is mainly via the feed so that as with sodium a considerable quantity of the total body potassium in the ruminant is found in the rumen. This has been estimated as 20% and offers a considerable buffer to any tendency to hypokalaemia. The percentage will vary however according to the nature and composition of the diet and the amount of sodium in the saliva (Lohman *et al.*, 1966).

The amount of potassium needed daily for cattle has been calculated for maintenance as 133 mEq/kg body weight/day (Pradhan and Hemken, 1968). A deficiency in the grazing animal is unlikely to occur as it has been estimated that dairy cows grazing lush pasture consume more than 300g potassium per day (Rook and Balch, 1962). This amount increases to 500g potassium per day on a diet of lucerne hay (Ward, 1966). The excretory mechanism appears able to cope with the relatively large amounts of potassium ingested by ruminants and it has been found that sheep for example may consume a 5% solution of potassium carbonate without developing a hyperkalaemia (Pearson *et al.*, 1949).

It has been suggested that there is no difference in the availability of potassium in the different feed stuffs since the availabilities of potassium in white clover and perennial rye grass are similar (Joyce and Rattray, 1970a). This may be an oversimplification of the position however, as the uptake of dietary potassium, as well as being influenced by the amount present in the diet, is also influenced by the dry matter content and by the total nitrogen intake (Paquay *et al.*, 1969).

The site of absorption of potassium varies according to its concentration in the gastro-intestinal tract contents and in the serum. At high intakes there is a net absorption of potassium from the stomach, a major net uptake by the small

intestine (principally the lower small intestine as there is a net secretion of potassium in the upper small intestine), and a net absorption from the large intestine (Smith, 1962; Grace *et al.*, 1974). The rate of absorption is said to be governed by the concentration gradient from the intestinal contents to the bloodstream so that in contrast to a net absorption at higher intakes, a net secretion may occur at very low intakes (Parthasarathy and Phillipson, 1953).

The amount of sodium in the diet can apparently alter the excretion pattern since on a high sodium diet 62-63% of oral potassium was excreted via the urine whereas on a low sodium diet faecal potassium rose to 53% of the total and total potassium excretion was elevated (Devlin and Roberts, 1963). This is a sodium conserving mechanism in the ruminant. However, when increasing amounts of sodium chloride are added to a cows diet and an upper limit for sodium excretion is reached, potassium excretion is also elevated to maintain plasma osmotic pressure (Elam and Autry, 1961). Irrespective of how high the dietary intake of potassium is there is apparently no effect on urinary sodium excretion (St Omer and Roberts, 1967). There is evidence that sodium and potassium may be inversely related in plasma (Lane *et al.*, 1968) and that this is probably an effect of aldosterone since it has been found that, during a sustained water diuresis, exogenous aldosterone caused a reduction in the sodium content of the urine followed sixty minutes later by an elevation of the potassium level (Sonnenblick *et al.*, 1961).

A number of factors affect serum potassium levels: -

a) Season

Neither Rowlands *et al.* (1974a) nor Ross and Halliday (1976) were able to confirm that season had any influence on serum potassium levels although the former authors observed wide fluctuations throughout the year.

b) Pregnancy

Pregnancy has been reported to have no effect on potassium level (Rowlands *et al.*, 1975). However in another report potassium level did vary with month of pregnancy (Lane *et al.*, 1968).

c) Lactation

Plasma potassium has been reported as falling with increasing milk yield (Lane *et al.*, 1968; Payne *et al.*, 1974). Hewett (1974) found the reverse, namely that high values were associated with high levels of milk production but surprisingly there was no effect with stage of lactation. Hewett believed this not to be associated with nutrition and no differences had been reported when feeding regimes were altered in an earlier investigation by Bartlett *et al.* (1953).

d) Nutrition

Despite the claim by Bartlett (1957) that altered feeding regimes caused no differences in plasma potassium levels, such effects have been reported by other workers (Belyea *et al.*, 1976). Differences due to this source resulted in potassium levels within the normal range. During a six month underfeeding trial it was found that potassium was held within fairly narrow limits (Roberts *et al.*, 1978).

e) Genetic Influences

It has been suggested that part of the variation in potassium levels observed between herds is due to genetic factors (Barber *et al.*, 1975). Kitchenham and Rowlands (1976) for example found that the serum potassium levels of Ayrshire cattle were lower than Friesians with the crossbred falling between the two. These same workers reported that there were differences in the mean potassium levels between groups of daughters from different sires and suggested that heritability of this trait was

relatively high. However Wiener and Field (1971) have reported the percentage of variation of serum potassium due to genetic effect to be zero and Healy and Falk (1974) report little difference between breeds. Nevertheless Simon *et al.* (1978) report the heritability of serum potassium to be 0.51.

f) Age

Some workers have reported a fall in potassium level in the serum with increasing age (Little *et al.*, 1977; Bide and Tumbleson, 1976), others claim there is no consistent change (Tumbleson *et al.*, 1973b; Healy and Falk, 1974), and still others record that levels are highest at intermediate ages (Lane *et al.*, 1968).

g) Diurnal Effect

On a short term basis most variation was associated with individual cow/day effects, i.e. different cows behaved differently on different days (Unshelm and Rappen, 1968). Although the hourly variations of potassium were either significant or highly significant their contribution to the total variation observed was small (5%). Unshelm and Hagmeister (1971) reported that the potassium concentration increased during the day till noon and then decreased reaching a minimum at 1600 hours. This is the inverse diurnal pattern reported for sodium (see previous section).

h) Stress

No differences in potassium levels were noted between animals which were subjected to the stress of trucking and those not so stressed (Healy and Falk, 1974; Crookshank *et al.*, 1976). Plasma potassium rose in starved sheep (Gitter *et al.*, 1975), and rose with acute cold exposure at $-20^{o}C$ (Sykes and Slee, 1969).

i) Sex

Similarly sex related differences have been both disclaimed (Healy and Falk, 1974) and claimed (Doornenbal, 1977; Stark *et al.*, 1978).

Hyperkalaemia is unlikely to arise in the adult bovine except in the case of a prolonged high level of intake associated with some degree of renal failure. In the young animal it can result during the terminal stages of diarrhoea (Bergman and Sellers, 1954; Roy *et al.*, 1959; Fisher, 1965). The calves became irritable and then finally were depressed with bradycardia present in 30% of them and arrythmias present in others (Fisher, 1965).

Sansom (1973) has stated that potassium deficiency on pasture forage is unlikely to occur. Nevertheless it may develop where grain cereals are used extensively for milk production (Pradhan and Hemken, 1968). Hypokalaemia however can arise for other reasons, e.g. during the early stages of diarrhoea and again in the later stages; when correcting dehydration, there may be a drain of potassium into the cell causing this effect (Fisher, 1965). In cases of experimental potassium deficiency animals had poor appetites, daily weight gains fell and there was a weight loss. Other symptoms included some degree of inanition, pica and hair licking (Devlin *et al.*, 1969).

Other possible effects of alterations in potassium level that have been observed include an inverse relationhip between potassium levels and growth rate (Payne *et al.*, 1970a, 1973) and a similar relationship to number of services to conception (Rowlands *et al.*, 1977b). Hewett (1974) also reported a depression in fertility when potassium levels were higher. Whether the relationship is cause and effect or simply a correlation between two events remains to be established.

The level of serum potassium in cattle has been reported to be 4.4 ± 0.9 mEq/l in Israel (Bogin *et al.*, 1974) and 5.0 ± 0.7 mEq/l in the United Kingdom (Payne *et al.*, 1973).

Magnesium

Seventy percent of total body magnesium is present in the skeleton, 29% in the soft tissue and 1% in the extra-cellular fluid; this is equal to about 2g magnesium (Wilson and Tribe, 1964). Some of the skeletal magnesium is available for mobilization in times of dietary inadequacy and that which is readily available is probably stored in the bony lattice (see section on calcium).

Taylor (1959) has shown that in bone, magnesium exists in two different forms as governed by its solubility in dilute acid; two thirds of the bone magnesium being readily removed and the balance is in a closer association with and possibly an integral part of the bone crystal structure. The amount that is available from skeletal reserves declines with age (Blaxter and McGill, 1956). In the immature animal this may be 30-60% whereas the adult may die from hypomagesaemic tetany with little or no alteration in bone magnesium level (Cunningham, 1936a, b; Allcroft, 1960).

Apart from bone, magnesium occurs chiefly intra-cellularly. Intra-cellular fluid contains approximately 36mg/100ml compared with the plasma concentration of 2.4mg/100ml. The mechanism maintaining this fifteenfold gradient in magnesium concentration is currently believed to be the existence of intra-cellular fluid magnesium in two forms: one bound with adenosine triphosphate and certain apo-enzymes, and a free labile form which is probably present at about the concentration in the extra-cellular fluid (Stevenson and Wilson, 1963). In spite of the fact that there is such a large amount of magnesium in tissue, and this is in a state of free interchange with the magnesium in the extra-cellular fluid (Rogers and Mahan, 1959), only 4% of tissue magnesium is labile and able to act as a body reserve (Care, 1960).

In vitro studies have shown that magnesium is required to activate many enzyme systems and other data indicates that it plays a significant role in intra-cellular catalysis (Wacker and

Vallee, 1964). Physiological studies have demonstrated that injection of magnesium salts results in peripheral muscular paralysis. The neuro-muscular junction seems to be the site of action. A low concentration of magnesium, particularly a low magnesium:calcium ratio, potentiates the release of acetylcholine and it is possible that a low concentration of magnesium could produce tetany by this means (Blaxter *et al.*, 1954a). Excess magnesium results in depression of the central nervous system leading to respiratory failure and cardiac arrest.

Absorption of magnesium is low in cattle and its apparent availability is 20-25% compared with 39% in monogastrics. The calf fed on milk resembles this monogastric pattern and the ability to absorb magnesium declines with maturity (Smith, 1959a, b). Generally there seems to be a net absorption in the forestomachs and stomach region (Care, 1967; Grace and Macrae, 1972; Grace *et al.*, 1974; Axford *et al.*, 1975; Strachan and Rook, 1975; Horn and Smith, 1976; Tomas and Potter, 1976a), a net secretion in the anterior portion of the small intestine (Perry *et al.*, 1967; Kay and Pfeffer, 1970; Grace *et al.*, 1974; Axford *et al.*, 1975), and a net absorption from the balance of the small instestine (Smith, 1962; Care and van T'Klooster, 1965; Perry *et al.*, 1967; Kay and Pfeffer, 1970; Grace and Macrae, 1972) and large intestine (Smith, 1962; Perry *et al.*, 1967; Grace *et al.*, 1974). Absorption does not normally occur from the rumen (Stewart and Moodie, 1956; Phillipson and Storry, 1965) but there is absorption from the omasum and abomasum (Horn and Smith, 1976), the holding of the mineral in the milk clot facilitating this in the young calf. Following oral dosing the maximum effect on plasma is 8.2 hours later (McAllese *et al.*, 1961).

While magnesium is largely absorbed by passive diffusion (Scott, 1965), and Care and van T'Klooster (1965) found that absorption only occurred when there was a concentration gradient in its favour, more recent work (Brown *et al.*, 1977; Martens *et al.*, 1978) indicates that absorption of magnesium from the abomasum is an active process. It would appear there-

fore that the absorption process differs according to the site at which it takes place and according to the relative concentrations of magnesium in the plasma and the gastro-intestinal tract. Binding of the mineral (Smith and McAllen, 1966; Storry, 1961) in the small intestine and, in cattle, the large quantity of water retained in the ingesta both contribute to the low level of absorption that takes place (Smith, 1969).

Generally the diet contains adequate magnesium (Rook, 1963), and Payne (1970) stated that most cases of hypomagnesaemia could not be explained on the grounds of a simple deficiency as so-called'tetany -prone pastures'often contained more than adequate magnesium. Nevertheless there are variations in the magnesium content of herbage. Young rapidly-growing grass resulted in lower serum magnesium than older feed (Rook and Balch, 1958; Smyth *et al.*, 1958; Kemp *et al.*, 1961; Michael, 1962). This is the result of magnesium in the plant increasing slowly during the growing season so that there are low points in the magnesium content of the pasture in early spring and late autumn (Simesen, 1970). Short forage has been found to have lower magnesium than tall forage (Barrentine, 1966). It has been reported (Joyce and Rattray, 1970b) that on an *ad lib* diet, 2.6g/day magnesium was absorbed from white clover as opposed to 1.8g/day magnesium from a perennial rye grass diet. The deliberate introduction of clover and certain herbs to tetany-prone pasture has been effective in reducing the tetany proneness of the pasture (T'Hart, 1960).

Wilson and Grace (1978) compared cows fed "Matua" and "Tama" strains of ryegrass with cows fed mixed perennial ryegrass/ clover pasture. They found that plasma magnesium concentrations were significantly lower in cows fed the annual ryegrass species. Mineral management of the pasture, i.e. the fertilization programme, however may be more important than changes in the botanical composition (Michael, 1962) in respect to magnesium availability.

The form of the magnesium in the soil to some extent governs the availability of the soil magnesium to the plant. In one series of trials (McNaught *et al.*, 1973a, b) magnesium oxide was found to give a greater increase in forage magnesium content than a variety of other compounds and in another trial (McIntosh *et al.*, 1973) it was reported that magnesium sulphate resulted in a greater response than magnesium oxide. In general it is considered that the plant magnesium content is related to the level of exchangeable magnesium in the soil and that plant magnesium uptake is modified considerably by levels of other exchangeable cations (Metson, 1974).

Variations between pastures in their ability to produce hypomagnesaemic tetany have been explained by differences in the availability of the magnesium content (L'estrange and Axford, 1966). It has been reported that the magnesium content of concentrates is 10-40% available while the magnesium content of pasture is 5-35% available (Kemp *et al.*, 1961). Rook *et al.* (1958) found differing percentages of magnesium absorbed between typical stall rations in the U.K. and fresh cut herbage even though a similar amount of magnesium was present in each. In some cases the solubility of magnesium in normal and tetany prone pastures has been compared and no difference has been reported (Parr, 1957b) whereas van T'Klooster (1965) has shown the reverse, i.e. a reduced solubility of magnesium in grass which was tetany prone. This may be associated with binding of magnesium to food fibre and higher fatty acids present in plants (van T'Klooster, 1969). This author also suggested that magnesium was absorbed in an ionic state from some foods such as hay and concentrates whereas electrostatic differences on grass diets prevent this happening. It could explain the need for two mechanisms of absorption and why on some diets, as has been explained later, magnesium absorption appears to be energy dependant.

The balance of other minerals within the grass has been the subject of a fairly intensive study. Smyth *et al.* (1958)

in one of the earlier studies reported that potassium excess on its own was not effective in reducing the absorption of magnesium but where high nitrogen content was involved also, potassium did reduce absorption, an effect which could be overcome by increasing the magnesium content of the diet. Other workers (Kunkel *et al.*, 1953; Fontenot *et al.*, 1960; Suttle and Field, 1969) found on various diets that increasing the potassium intake caused a proportional decrease in the absorption of magnesium and Dishington (1965) reported that potassic fertiliser on pasture tended to make the pasture more tetany prone. This is apparently related to the availability of the magnesium content (Bartlett *et al.*, 1957; Smyth *et al.*, 1958) and has been suggested as one of the mechanisms by which the magnesium in young rapidly growing grass is less available: that is during this early stage of growth young grass has a higher potassium content than when it is more mature (Kemp *et al.*, 1966). The effect is exacerbated if it is associated with a lower magnesium content in the diet or under-nutrition (Suttle and Field, 1969).

It has also been suggested that plant magnesium uptake is strongly influenced by competition for uptake with potassium, hence some of the effect of potassic fertiliser is probably going to be a lowering of plant magnesium (Blaxter *et al.*, 1960; Care *et al.*, 1967). Many workers (Kunkel *et al.*, 1953; Kemp *et al.*, 1961; Ward, 1966; Suttle and Field, 1969; Lentz *et al.*, 1976; Tomas and Potter, 1976b) have reported a depression in magnesium absorption or serum magnesium following potassium supplementation of the diet.

Another dietary factor which is considered important in respect to magnesium availability is dietary nitrogen. Early workers (Bartlett *et al.*, 1957; Ender *et al.*, 1957; Smyth *et al.*, 1958) indicated that heavy fertilization with inorganic nitrogen affected the incidence of hypomagnesaemic tetany though Smyth *et al.* (1958) found this only occurred when associated with a high potassium fertilization level

as well as nitrogen fertilization. It was suggested that this was associated with the high protein content of young rapidly growing grass (Kemp *et al.*, 1961). The protein content of pasture did tend to be higher after nitrogenous fertiliser application (Dishington, 1965) but how this exerted its effect was not clear. The suggestion has been made that high rumen ammonia may be the cause (Head and Rook, 1955; Henry *et al.*, 1977). If there is sufficient protein to cause free ammonia and raise the pH, the solubility of the magnesium decreases and there is a decreased magnesium utilization, i.e. there is a direct effect on absorption (Lomba *et al.*, 1968). Other workers such as L'Estrange and Axford (1966) concluded that the nitrogen treatment of forages neither affected the magnesium availability of the forage nor the serum magnesium level of the ewes. Grace and Macrae (1972) noted that a protein supplement had no effect on the extent or site of magnesium absorption and Sanwal *et al.* (1975) found that absorption was enhanced when the protein level of the diet was increased.

Yet another element which could be associated with lactation tetany is calcium. Kemp and T'Hart (1957) carried out a survey in one area of the Netherlands and demonstrated a positive correlation between the incidence of hypomagnesaemic tetany and the potassium/calcium + magnesium ration (K/Ca + Mg) of the herbage. Butler (1963) confirmed that the incidence of hypomagnesaemic tetany was positively correlated with the above ratio but pointed out that there was no correlation between the ratio and the incidence or severity of hypomagnesaemia in clinically normal cows. The relationship of K / Ca + Mg in the pasture and the occurrence of hypomagnesaemic tetany held in some areas and in some years but was not universally applicable (Seekles, 1964). The effects of dietary calcium are variable being beneficial according to some reports but not in others. Thus Care and van T'Klooster (1965) found that magnesium absorption from ileal loops was depressed if there was a higher than normal calcium in the perfusing solution, while binding of calcium in the diet in a non-absorbable form improved magnesium

absorption (van T'Klooster and Care, 1966). Nel (1976) also found that poor magnesium retention was responsible for hypomagnesaemia and that reduction of both phosphate and calcium in the diet of cattle enhanced magnesium retention and increased urinary output. Others advanced evidence in the reverse direction by reporting that serum magnesium was lowest on low calcium high phosphorus rations and that no effect was observed on a high calcium ration (Wise *et al.*, 1963) while Lomba *et al.* (1968) suggested that increasing calcium as well as magnesium was more effective at raising the serum magnesium level.

Phosphate has also been claimed to have some effect on serum magnesium level. Dutton and Fontenot (1967) found serum magnesium was depressed more on low magnesium rations when inorganic phosphate replaced the organic phosphate supplement. It was suggested that this was due to the formation of insoluble magnesium complexes which lowered absorption (van T'Klooster, 1969). Noller *et al.* (1977) however found phosphate supplementation had no effect on serum magnesium level.

Fertilisers incorporating sulphate have been implicated in the proneness of pasture to be tetany-producing but so far no experimental work has been carried out to verify this (Ender *et al.*, 1957). Pastures managed in such a fashion as to induce tetany proneness did cause significant alterations in sulphur level in plants (Dishington, 1965). In this same trial the author commented on differences in the sodium level of pastures that were tetany prone as it had earlier been found that such pastures had significantly lower magnesium and sodium levels (Butler, 1963). Ivins and Allcroft (1969) on the other hand found that sodium supplementation had no effect on the serum magnesium level.

Besides minerals, various other plant components have been found to apparently render the magnesium content of the diet unavailable. Amongst these are naturally occurring plant lipids. It has been found that the addition of animal fats

to the diet increases magnesium excretion in the faeces and decreases magnesium availability (Kemp *et al.*, 1966; Lomba *et al.*, 1968). The long chain fatty acids are thought to chelate the magnesium as insoluble magnesium soaps. It is also significant that higher pasture protein is usually accompanied by higher pasture fatty acids (Kemp *et al.*, 1966; Molloy *et al.*, 1973).

Despite one report which did not support the concept that a lower dietary energy intake is associated with poor utilization of magnesium in lactating beef cattle (Fordyce *et al.*, 1974) there is considerable evidence that a low carbohydrate intake does result in a low utilization of magnesium in some animals. Reduction of calcium and magnesium levels in serum was found during starvation experiments in dairy cows (Dale *et al.*, 1954; Swan & Jamieson, 1956b; Robertson *et al.*, 1960; Pehrson, 1963; Herd, 1966) and where some degree of hypomagnesaemia already existed a short period of starvation was often the triggering factor for the onset of tetany. Rook and Storry (1962) and Dishington (1964) suggested that as the greater part of the carbohydrate in the ruminant diet is converted to volatile fatty acids and absorbed in this form, there may be a deficiency of readily soluble carbohydrate leading to a poor absorption and reduced availability of magnesium. In a series of trials, the feeding of molasses to fasted sheep (Cunningham, 1936b), starch to grazing dairy cattle (Wilson *et al.*, 1969) and sucrose to sheep (House and Mayland, 1976) resulted in elevation in serum magnesium in the treated compared with the control animals. This is apparently the result of an increase in magnesium availability (House and Mayland, 1976; Madsen *et al.*, 1976). Other workers suggested it was the high protein, low energy diet that was important (Metson *et al.*, 1966) and it was observed that as starch levels decreased, rumen ammonia levels increased (House and Mayland, 1976). This was summed up in the statement of Wilcox and Hoff (1974): "winter leaching and soil temperature make the ammonium ion the principal source of nitrogen to the plant during the early spring period.

The absorption of ammonium by the plant results in a greatly reduced uptake of calcium and magnesium with little effect on potassium. This results in a high amide content in the plant with depletion of carbohydrates. High rumen ammonia, increased pH, depletion of carbohydrates and a further reduction of an already low magnesium follows."

Burau and Stout (1965) found that high levels of trans-aconitic acid were present in samples of range grass where hypomagnesaemic tetany had occurred periodically and that in fact 47% of grasses under range conditions were trans-aconitic acid accumulators (Burau and Stout, 1965; Stout *et al.*, 1967). Hypomagnesaemic tetany has been induced in a high percentage of cattle by drenching with citrate and/or trans-aconitate (Bohman *et al.*, 1968). However while dosing sheep with progressively increasing doses of potassium trans-aconitate caused a significant depression of serum magnesium, dosing with trans-aconitic acid did not significantly alter the serum magnesium in a trial by Camp *et al.* (1968). Other workers have found that there was no effect on the serum magnesium level as a consequence of dosing sheep with a single large dose of trans-aconitate by stomach tube (Wright and Wolff, 1969). A further trial produced symptoms which resembled field cases of hypomagnesaemic tetany by the administration by stomach tube of potassium chloride and either trans-aconitic acid or citric acid (Brown *et al.*, 1977). Potassium may have been the more important factor in inducing the symptoms in this case.

Some animals have a low absorptive capacity apparently independant of other factors inhibiting magnesium absorption with the result that serum magnesium is slow to respond even to oral dosing (Hemingway *et al.*, 1963). In most cases a high magnesium diet results in a high plasma magnesium (Chicco *et al.*, 1973).

The form in which the magnesium is available is quite important. It has been found for example that magnesium acetate actually raised blood magnesium level more than

magnesium oxide dosing even though the magnesium content of the oxide was higher (Rogers and Poole, 1976). This still did not prevent hypomagnesaemia even though no clinical case was observed. Larvor (1976) found that after changing from normal to frozen tetany prone grass, there was a small drop in blood magnesium and a significant decrease in urinary magnesium indicating a marked reduction in magnesium absorption, a rather unexpected finding as it has been found that generally fewer cases of hypomagnesaemic tetany occur after a frost (Inglis, 1960). Magnesium content of hay on the other hand seems to be relatively more available than in feedstuffs such as green grass (Crawford, 1967; Church, 1971).

Hutton *et al.* (1965) found during magnesium balance studies that milking cows consumed magnesium at the rate of 0.1g/kg live weight daily; 80% of the magnesium ingested was excreted in the faeces, 12% in the urine and 8% either in milk or retained in the body. Absorption seems to be greater at 3 weeks of age than at 16 weeks of age - thereafter it remains relatively constant on steady diets but changes with dietary changes (Smith, 1958).

The magnesium balance is said to be zero when the amount of magnesium excreted in the faeces, milk, urine and any other route equals the amount contained in the diet. To stay in balance cows yielding 2-6 gallons milk per day need to absorb 3.5-5.8g Mg per cow per day (Rook *et al.*, 1958).

Magnesium is lost to the body by three routes. The concentration of milk magnesium is 9-16mg/100ml and up to 3g per day may be lost by this route (Blaxter and Rook, 1955; Parr, 1957a,b; Field, 1960; Robertson *et al.*, 1960). The magnesium concentration of milk varies from cow to cow but is relatively constant for each individual regardless of their serum magnesium level.

Endogenous loss in the faeces is widely variable but there does appear to be an obligatory loss of approximately 2g per day minimum for the average dairy cow (Blaxter and McGill,

1956; Rook and Balch, 1958; Storry and Rook, 1963). Payne (1977) has pointed out that these two routes amount to a minimum obligatory loss of approximately 5g per day; this is over twice the amount in the extracellular reserve and occurs at a time when input is in a relatively short supply. Uninterrupted absorption from the alimentary tract is thus of great importance at this time.

The third route of loss of magnesium is in the urine. Urinary excretion is very adaptable and in the presence of hypomagnesaemia it may fall to virtually zero (Storry and Rook, 1963). Even when the serum level was unaffected urinary magnesium level fell when the animal was in a negative magnesium balance (Rook and Balch, 1958; Rogers, 1979). This mechanism is utilised in studying magnesium absorption but the control of it has not been explained. Some cattle are able to adapt to a low dietary intake of magnesium (Rook and Balch, 1958) and/or maintain serum levels when intake is normal but other factors interfere with absorption (McConaghy *et al.*, 1963). Other cattle are tetany-prone (Hjerpe, 1968), a state which may be recognised by the relative instability of their serum magnesium level (Payne *et al.*, 1970a).

Similar pathways appear to operate for calcium and magnesium in respect to their homeostatic control. Following parathyroidectomy there is an immediate fall in serum magnesium level followed later by a return to normal (Payne and Channings, 1964). Buckle *et al.* (1968) evaluated the effect of plasma magnesium concentration on the parathyroid gland by perfusion of an isolated parathyroid gland in five goats and one sheep. In each animal they observed that the concentration of parathyroid hormone in the effluent plasma diminished when the concentration of magnesium was raised or increased when the concentration of magnesium was lowered so that some feedback effect exists. Calcitonin has been found to be antihypermagnesaemic in both the goat (Barlet *et al.*, 1971) and the bovine (Barlet, 1971). Magnesium infusion in the bovine induces a significant hypocalcaemia

and hypophosphataemia suggesting that it has triggered a release of calcitonin.

Other hormones have been found to effect serum magnesium levels but in the absence of known feedback mechanisms it is not certain how significant they are in homeostasis. Hyperthyroidism leads to hypomagnesaemia and hypothyroidism to hypermagnesaemia (Hanna, 1961; Inskeep and Kenny, 1968). However it is unlikely that hypersecretion of the thyroid is a primary factor in clinical hypomagnesaemia (Todd and Thompson, 1962). Aldosterone has also been found to effect serum magnesium level as hyperaldosteroneism in man is associated with a negative magnesium balance and a similar finding has been obtained in sheep (Care and Ross, 1963). In adrenal insufficiency as in adrenalectomised animals or in human patients with Addison's disease, the reverse occurs. No effect of the serum magnesium level could be found on the rate of aldosterone secretion (Care and McDonald, 1963).

It is also reported (Care and Ross, 1963) that desoxycorticosterone acetate brought about a reduction in magnesium retention, without affecting the urinary excretion of magnesium. The use of radio-active tracers demonstrated that all magnesium in soft tissue was in a fairly rapid state of exchange (Rogers and Mahan, 1959). It was further demonstrated that only 4% of tissue magnesium was labile. Little transfer can therefore be expected in cases of low intestinal absorption if virtually no reserves exist.

Finally insulin may be involved in magnesium homeostasis since an insulin injection was followed by a fall in plasma magnesium brought about by a mechanism which is supposed to be increased tissue uptake (Persson and Luthman, 1974b). How valid this is remains uncertain as glucose infusions do not alter plasma magnesium. More recently Lentz *et al.* (1976) has found a possible effect of potassium by this means. Thus potassium infused intra-ruminally has been found to depress serum magnesium even though uptake of magnesium remained about the same. The potassium and insulin levels

in the serum were elevated and as long as the increased insulin level remained, even though potassium had returned to normal, the serum magnesium stayed depressed.

A number of other factors influence serum magnesium levels: -

a) Season

No clear pattern has been established. Thus Ross and Halliday (1976) and Claypool (1976) have reported lowest levels of magnesium in the serum in the spring and autumn and in the spring respectively while Payne (1972b) found that this occurred in the winter. Payne and coworkers indicated that this was the result of a summer magnesium-supplementation programme (Payne *et al.*, 1974). Studies on the influence of temperature have not clarified the position since both high temperature (Yousef and Johnson, 1965) and low temperatures (Sykes *et al.*, 1969) have been reported to reduce serum magnesium. In the former investigation the serum magnesium levels were high and would have been difficult to sustain. Mears and Groves (1969), to confuse the matter further, claimed no reduction due to the cold at 2°C but rather a slight increase. A possible explanation for this is that protein in the diet may increase in the cold and this increased protein can in turn lead to the increased absorption of magnesium (Sanwal *et al.*, 1975).

b) Pregnancy, Parturition and Lactation

It has been found that the concentration of magnesium varies significantly with the stage of both pregnancy and/or lactation with the most significant changes being confined to the three months on either side of calving (Rowlands *et al.*, 1975). Generally magnesium has been found to be low in non-lactating cows (Payne *et al.*, 1973) during which time the plasma magnesium in the foetus is higher than in the mother (Wilson *et al.*, 1977). Close to calving the maternal magnesium tends to rise (Moodie *et al.*, 1955; Wilson *et al.*, 1977) and to

peak 24 hours after calving (Moodie *et al.*, 1955). In one trial however, the magnesium level decreased at calving and returned to normal three days later (Kirchner *et al.*, 1977), a change which could be explained in terms of a reduced food intake during the peri-parturient period. Generally the effect of lactation has been reported as small with the magnesium level in the serum tending to rise with increasing milk yield (Payne *et al.*, 1973; Rowlands *et al.*, 1975).

c) Nutrition

In the short term there is an increase in serum magnesium level and an increase in urinary excretion after feeding on a magnesium sufficient diet (Stacy, 1969; Coggins and Field, 1976). While Belyea *et al.* (1975) have reported no consistent differences due to feeding regime and that serum magnesium level was held within fairly narrow limits during a six month underfeeding trial (Roberts *et al.*, 1978), there are a large number of papers that confirm that serum magnesium falls in undernutrition whether the animals are lactating or not (Blaxter *et al.*, 1954a, b; Robertson *et al.*, 1960; Lomba *et al*, 1972, Economides *et al.*, 1973; Rogers *et al.*, 1977).

d) Genetic Influence

Breed differences have been reported (Bartlett *et al.*, 1957; Kitchenham and Rowlands, 1976) and heritability groupings between sires and their daughters or dams and daughters have been confirmed (Henricson *et al.*, 1975). There is a greater constancy in magnesium levels between members within a twin pair than between twin pairs (Kay *et al.*, 1976) and the heritability has been calculated as 0.37 (Simon *et al.*, 1978).

e) Age

The magnesium level generally falls over the first three or four months of age and then remains fairly constant after that (Smyth *et al.*, 1977; Kitchenham and Rowlands,

1976).

f) 'Stress'

There was no change in magnesium level due to trucking, weaning or both (Crookshank *et al*, 1976). The level showed a tendency to fall in *Ostertagia* parasitized calves (Waymach and Torbet, 1969).

The most visible effect of hypomagnesaemia in the animal is increased uncontrolled muscular twitching which may progress to tetanic convulsions. The clinical disease is commonly known as grass staggers or lactation tetany. It has been claimed that a hypocalcaemia is necessary before hypomagnesaemia develops into a clinical tetany (Hemingway *et al.*, 1965) but in other surveys 50% of the cattle suffering hypomagnesaemia were normocalcaemic (Todd, 1969).

The critical factor in the initiation of tetany is the level of magnesium in the cerebro-spinal fluid (CSF), a hypothesis first put forward by Meyer and Scholz (1972). Magnesium is thought to be accumulated in the CSF by an active transport mechanism in the choroid plexus and is removed by bulk filtration through the arachnoid villi. Levels of magnesium in the CSF are held within strict limits despite wide variations in the serum. Pauli and Allsop (1974) found that the CSF level of magnesium was more closely related to the onset of tetany than was the serum level and by perfusion of the CSF space with artificial CSF low in magnesium they were able to produce tetany (Allsop and Pauli, 1975a). This has been substantiated from field cases where tetany only develops in those hypomagnesaemic cows which have a low CSF concentration of magnesium (Allsop and Pauli, 1975b).

'Stress factors' such as sudden changes in management, inclement weather, transportation and the onset of oestrus have all been suggested as placing additional stress upon the animal that may already be in negative magnesium balance thus precipitat-

ing an attack of clinical tetany (Swan and Jamieson, 1956a). This stress could be mediated by an increase in lipolysis which is accompanied by the accumulation of magnesium in adipose cell membranes (Larvor and Rayssiguier, 1977), and it has been found possible to prevent hypomagnesaemia by treating animals with an antilipolytic agent.

Other effects of hypomagnesaemia that have been reported include a fall in energy retention associated with the increased muscular activity (Blaxter and Rook, 1955), a condition of 'leatherbag' or ventral oedema of the udder (Hicks and Pauli, 1976) and a reduction of milk fat production (Thompson, *et al.*, 1974-1975; Bogin *et al.*, 1975; Young and Rys, 1977; Young *et al.*, 1978) although non-significant responses to magnesium supplementation have also been recorded (Wilson and Grace, 1978; Maung, 1980). Wilson (1980) from a trial comparing magnesium supplementation by several routes obtained significant responses only through oral dosing; he concluded that this enhanced ruminal fermentation and throughput of organic matter, which in turn, resulted in greater food intake and milk fat production. Another argument that has been advanced to explain production responses is that cows with subclinical hypomagnesaemia are less aggressive, and their access to the better quality food that may be available is reduced (Church *et al.*, 1978).

It is not surprising, in view of what has been reviewed above, that Smyth (1976) came to the conclusion that magnesium is a useful parameter for inclusion in a metabolic profile and low values for a group mean indicate a definite need for supplementation.

Hypermagnesaemia is unlikely to result naturally because of the excretory mechanisms whereby fairly large quantities are passed out in the faeces and urine (Kunkel *et al.*, 1953). An oral overdose may result in colic and diarrhoea in cattle (Young *et al.*, 1979), an intravenous overdose causes initially a respiratory embarrassment, some A/V conduction depression

and ultimately death through cardiac arrest (Bergman and Sellers, 1954).

The level of serum magnesium values vary with different investigations but in most cases normal values seem to fall into the range 2.0 to 2.5mg/100ml (Bogin *et al.*, 1976; Ross and Halliday, 1976; Church *et al.*, 1978).

Calcium

Together with phosphorus, calcium is largely contained in the skeletal structures. In mammals, 36% of ash from adult bone is calcium and this constitutes 99% of the total body calcium (Simesen, 1970). The very minor portion present in body fluids is however vital, being critical for blood coagulation, capillary and cell membrane permeability, the transmission of nerve impulses and neuromuscular excitability. In tissues the presence of calcium is essential for the activity of a number of enzyme systems, including those responsible for the contractile properties of muscle where it appears that a calcium - magnesium - ATP complex provides the substrate for the enzyme ATP - ase which liberates the energy for contraction. Ionic calcium is one of several ions (potassium, sodium, magnesium and hydrogen) that are concerned with the maintenance of normal neuro-muscular activity (Church, 1971). Possibly because of its vital part in the body fluid composition, the body has developed a relatively effective homeostatic mechanism for maintaining serum calcium levels even where nutritional deficiency exists.

Ultimately the amount of calcium in the feed will determine the amounts of calcium absorbed from the gut. In cattle it seems difficult to provoke a drop in serum calcium by feeding calcium-deficient diets (Groenewald, 1935). Most investigations appear to have been related to attempts to prevent or reduce the incidence of milk fever (Goings *et al.*, 1974; Wiggers *et al.*, 1974). The latter workers did promote a fall when feeding a diet that met the energy, protein and phosphate requirements but was low in calcium. This fall persisted for only four days, then rose and stabilized at a normal level. The delay would appear to be related to the activation time of the calcium resorption enzymes before they became effective in restoring the calcium levels.

An elevation in serum calcium frequently follows an excessive intake, an effect which may increase the incidence of milk

fever. Black *et al.* (1973) for example reported that high dietary calcium before calving resulted in an elevated serum calcium, that this decreased the ability of the animal to maintain serum calcium levels at calving, and that there was an increase in the number of milk fever cases. Others (Chicco *et al.*, 1973; Black and Capen, 1973; Hewett, 1974) obtained the same effect but found that if the high calcium diet was fed for only a short time, then serum calcium was not elevated; they indicated that serum calcium level was not a good indicator of dietary intake, agreeing with the earlier observations of Payne *et al.* (1970b). A number of other workers have similarly indicated that high dietary levels of calcium have little influence on serum levels (L'Estrange and Axford, 1966; Dunham and Ward, 1971; Ekman, 1975) although Luthman and Persson (1977) have reported that serum calcium fell when cattle were fed a pelletted ration rich in calcium.

Very little has been written on the availability of calcium in various feeds. In pasture white clover has been reported as having 55% more calcium than perennial ryegrass (Beeson and Perry, 1975) but calcium availability is very similar (Joyce and Rattray, 1970b). Young rapidly growing grass was reported by Michael (1962) to lead to lower serum calcium levels than when cattle were fed older grass. This may not have been the consequence of calcium content of the feed but could have resulted from the young grass, with its higher protein levels, leading to increased ammonia production in the rumen and a shift towards alkalinity in the rumen pH. Apparently more calcium enters the calcium pool when the diet leads to more acid conditions in the rumen (Vagg and Payne, 1970).

The calcium : phosphorus ratio (Ca:P) is apparently important in respect to calcium uptake. Thus a high Ca:P has resulted in increased serum calcium levels according to Saarinen (1950) whereas Anderson *et al.* (1970) reported that a wide variation from a normal Ca:P of 1.3:1 resulted in a reduction in calcium entering the calcium pool. Others (Wiggers *et al.*,

1974; Jonsson, 1978) report that it is not the ratio that is important but rather the total amount of calcium present.

Magnesium content of the diet may also affect assimilation of calcium although the evidence is by no means clear. Chicco *et al.* (1973) found that high dietary magnesium and phosphate both interfered with calcium absorption, while Smith (1962) indicated that magnesium supplementation did not alter calcium absorption at all.

Some of the explanations for the conflicting findings reported in the literature almost certainly revolve around our understanding of the mechanisms that control absorption and excretion of this element.

It has been stated that calcium absorption is a passive process (Bronner, 1964) with the intake / output balance being controlled by increased absorption and decreased excretion in terms of need and vice versa (Luick *et al.*, 1957; Paquay *et al.*, 1968). Bone resorption and accretion complicate this simple picture however, by providing an important reservoir of calcium for the soft tissue calcium pool.

Calcium appears to be not absorbed from the rumen but primarily from the anterior third of the small intestine (Phillipson and Storry, 1965; Kay and Pfeffer, 1970). Perry *et al.* (1967) however states that absorption is greater in the lower small intestine while Chandler and Cragle (1962), although agreeing that virtually no absorption occurs in the rumen, define the abomasum as one of the chief sites of absorption and the middle third of the small intestine as the other. Grace *et al.* (1974) have since confirmed that some absorption of calcium takes place before the pylorus, a finding which could be important in young calves where milk is held for some time in the abomasum (Smith, 1962) thus favouring the absorption of calcium in

the young animal. The large intestine may act as an absorption site also (Smith, 1962), although this varies with the amount of endogenous secretion of calcium into the bowel according to van T'Klooster (1965).

Bile salts, bile acids and certain detergents alter the absorption of calcium from the diet and could explain some of the alterations in calcium availability on different diets (Webling and Holdsworth, 1965). Whether their secretion may be altered in time of need in the ruminant is not known. Altered secretion of bile salts might equally explain the inherited inability of some cows to assimilate calcium from the feed thus predisposing them to hypocalcaemia (Ward *et al.*, 1952; Manston and Payne, 1964).

The formation of relatively insoluble calcium salts in the intestine may reduce absorption. Thus in rats it has been found that oxalates form an insoluble calcium oxalate which is lost in the faeces (Talapatra *et al.*, 1948). In ruminants, the oxalates tend to be broken down by rumen fermentation but the breakdown could result in alkalosis which could upset the calcium metabolism indirectly. Bowel movement may also be important as hyoscine, which reduces bowel motility, can trigger an attack of milk fever in a parturient cow (Moodie and Robertson, 1961; 1962).

Braithwaite *et al.* (1970) found that the rate of transfer of calcium to the foetus and milk was increased from about 65 days pre-partum to peak at parturition or shortly after, thus confirming earlier statements that lactating cows utilised calcium more efficiently than non-lactating, and pregnant cows more efficiently than non-pregnant (Luick *et al.*, 1957; Payne *et al.*, 1963). After parturition animals are actually in a negative calcium balance and move into a positive calcium balance by increasing calcium absorption as lactation proceeds (Braithwaite *et al.*, 1969). This confirmed an earlier report of Payne (1964a) who indicated that the increase in demand of a cow changing from late pregnancy to lactation was 0.8g per hour. In late pregnancy cows are

only able to mobilise half this amount.

Vitamin D is a most important factor in the regulation of the absorption of calcium and improves the efficiency of calcium transport. It appears to have a direct effect on the mineralization of bone as well as absorption (Greenberg, 1945). This vitamin is also involved in the mechanism that balances blood and skeletal calcium as it has been found in cattle that massive doses will increase blood calcium levels (Hibbs and Pounden, 1955). Vitamin D itself does not appear to be active in this regard but instead is carried to the liver on alpha-2-globulin where it is converted to 2,5-dihydroxy-cholecalciferol (25HCC) which is the active metabolite (De Luca, 1969). This mechanism explains why both Vitamin D3 injection (Julien *et al.*, 1977) and 25HCC (Olson *et al.*, 1973) are effective in reducing the incidence of hypocalcaemia.

Young animals absorb calcium more efficiently and to a higher maximum level (Braithwaite, 1975b). There is a decline in efficiency of calcium absorption with age, most of which has occurred by the time the animal is two years old (Hansard *et al.*, 1957). Symonds *et al.* (1966) demonstrated the extent of this when they showed that the transfer rate of calcium through the readily mobilizable calcium pool decreased from 12.6g per day as a calf to 5.3g per day as an adult. This is probably a result of the decreased amount of calcium required for skeletal development. Bone turnover is higher in young animals with skeletal calcium being mobilized in times of calcium deficiency and reserves replenished when dietary calcium is plentiful by changing rates of resorption and accretion.

Other factors that interfere with the absorption of calcium include the toxin of *Escherichia coli* (Griel *et al.*, 1975) and calves infected with *Ostertagia* sp. have been reported as having lower calcium balances and lower serum calcium levels than uninfected controls (Waymack and Torbert, 1969). Although it is not known whether parathyroid hormone affects

calcium absorption in cattle there is an elevated faecal excretion after exogenous parathyroid hormone is administered to parathyroidectomised calves (Mayer *et al.*, 1967).

The endogenous calcium loss has been estimated at 4-7g daily (Visek *et al.*, 1953a; Comar *et al.*, 1953). This loss does not appear to be closely related to diet but can be reduced when total calcium in the diet is very low (Ramberg *et al.*, 1975). The source of the endogenous secretion appears to be primarily the upper small intestine (Perry *et al.*, 1967; Grace *et al.*, 1974) although there was some secretion in both the rumen and omasum reported in one trial (Chandler and Cragle, 1962) as well as the large abomasal secretion. This may be the mechanism by which apparent intestinal loss increases with age as Hansard *et al.* (1957) has shown that endogenous calcium in the faeces becomes appreciably greater as the animal gets older.

The kidney threshold for calcium is when the serum level rises to 6.5 - 8.0 mg/100ml; below this little is excreted in the urine (Simesen, 1970). There is always some calcium lost by this route even where a negative calcium balance exists, but there is no evidence of tubular secretion (Wesson and Lauler, 1959). Kidney excretion is possibly important in controlling hypocalcaemia.

Mammary secretion in the lactating cow constitutes a major source of calcium loss and it has been assessed that one litre of colostrum contains as much calcium as the calcium content of the total plasma (Ramberg *et al.*, 1975). The amount of colostrum secreted, and therefore the amount of calcium drained by this mechanism, increases with each successive lactation (Payne 1964b). A fall in serum calcium has been reported in the peri-parturient period (Godden and Allcroft, 1932).

Another major outflow of serum calcium is to bone, and calcium deposition in bone is the largest outflow from the circulating calcium pool in the non-lactating cow. It varies

in rate according to size and age, reaching a peak rate at about six months of age and a peak amount at about fourteen months of age (Church, 1971).

Alterations in bone metabolism do occur and there is a slow down with increasing age (Hansard *et al.*, 1957). Since acidosis and alkalosis stimulate and slow down bone metabolism respectively the acid/base balance of the diet and the animal may also be important (Greenberg, 1945; Thomas *et al.*, 1967). Lactation causes a high demand on skeletal reserves and in some cases cows may not be able to recoup skeletal reserves during the non-lactating period even though they are on an adequate calcium diet (Manston and Payne, 1964). The reverse holds true and even on high lactation levels not all cows lose skeletal reserves (Paquay *et al.*, 1968). This appears to be a function of the individual.

The most important changes in bone metabolism occur at the time of calving and the initiation of lactation. There is a fall in bone accretion rate of approximately 4.7g per day from two weeks before to two weeks after calving (Sansom, 1969a, b). Bone accretion is then increased two weeks to two months after calving at a time when calcium demand for lactation is rising and cows are in a negative calcium balance. This is part of a general response of bone to lactation and indicates the increased mobility of calcium at this time.

There are two components which prevent serum calcium falling below normal levels - the first is a simple chemical equilibrium between a labile fraction of the bone mineral and plasma. This maintains the plasma calcium, except in times of excessive demand, at approximately 7.0 mg/100ml and is independent of hormonal control (McLean and Urist, 1955). The other mechanism, which functions by a negative feedback system, is dependant on parathyroid hormone increasing the resorption of cancellous bone and increasing phosphorus excretion in urine to maintain or decrease serum phosphate

level and to maintain or increase serum calcium level (Mayer *et al.*, 1968). This mechanism appears quite specific for calcium as it has been found that if serum calcium is unaltered then a variety of factors including changes in serum inorganic phosphate, Ca:P in diet and serum, and the presence or absence of Vitamin D in the diet do not alter parathyroid secretion rate (Roth *et al.*, 1968). The one possible exception to calcium being the direct stimulus for parathyroid secretion comes from a trial where there was a response to both serum calcium level and to serum calcitonin level (Du Fresne and Gitelman, 1972). Generally the stimulus for the secretion of parathyroid hormone is a drop in the level of circulating calcium (Parsons and Robinson, 1972) with a rise in serum calcium following. The most common time for this in cattle is in the periparturient period in response to the drain of calcium to meet the demands of colostrum. At this time it has been found that there is an elevation in circulating parathyroid hormone to a plateau which is achieved at 3-8mg/100ml serum calcium. The mechanism may function even if bone resorption is impaired (Mayer *et al.*, 1968).

Treatment with intravenous calcium solution induces a hypercalcaemia which is followed by a fall in parathyroid level (Blum *et al.*, 1974). In cases of parathyroidectomy there is a marked fall in serum calcium level (Stott and Smith, 1957; Williams and Stott, 1966) while exogenous parathyroid hormone causes a hypercalcaemia, the response being quicker with a purer product (Rasmussen and Westall, 1956); this response of elevation of serum calcium to increased parathyroid hormone secretion apparently does not occur in the parturient cow (Jackson *et al.*, 1962). When calcium is chelated there is an elevation in circulating parathyroid hormone within fifteen minutes (Ramberg *et al.*, 1967) and there is increased activity in the parathyroid gland in paretic cows (Mayer, 1970; Hibbs *et al.*, 1970). Furthermore blood transfused from paretic cows to calves caused a hypercalcaemia in the recipient (Nurmio, 1968).

As there did not seem to be any problem with parathyroid secretion in hypocalcaemic cows Kronfeld (1968) theorised that protein carriers, or enzymes, or both, in bone cells or intestinal mucosa, were less responsive than in normal cows.

To confirm that bone is the site of the apparent breakdown in parathyroid function in the hypocalcaemic animal, a model based on calcium tracer and kinetic studies was constructed (Ramberg *et al.*, 1970). This revealed that virtually no calcium from bone appeared in the bloodstream until two weeks post-partum and in the meantime intestinal absorption appeared to be enhanced. Thus there is a refactory period in the bone resorption mechanism in the immediate post-partum period. This occurs in all cows and hence may not necessarily be the cause of hypocalcaemic paresis; nevertheless those cows which develop the condition may have relatively more refactory bone or poorer intestinal absorption.

As changes in the serum calcium level in hypo- and hypercalcaemic cows were not adequately explained by parathyroid hormone withdrawal a new hypocalcaemic hormone was proposed (Copp *et al.*, 1962; Copp and Cameron, 1962). The likelihood of its existence was demonstrated by the development of a hypocalcaemia following perfusion of an intact thyroid gland with a calcium rich perfusate (Copp and Cheney, 1962); the substance had been found to be present in parturient bovine plasma but not non-parturient bovine plasma (Ochs *et al.*, 1964; Jackson *et al.*, 1970). The name calcitonin was suggested for this hormone and it has been found to be localised in the parafollicular cells of the thyroid parenchyma (Bussolati and Pearse, 1967). One suggested mode of its action is that it causes a net transfer of calcium from blood to bone (MacIntyre *et al.*, 1967) though it has also been suggested that it simply inhibits bone resorption (Aliapoulios *et al.*, 1966). More recently there has been a suggestion that calcitonin exerts its hypocalcaemic effect by facilitating a more rapid entry of calcium into all soft tissue cells (Parsons and Robinson, 1972). As well as

causing hypocalcaemia, calcitonin also causes a hypophosphataemia brought about by increased urinary excretion of phosphate.

The stimulus for the secretion of calcitonin appears to be hypercalcaemia. This has been demonstrated in other species (Care *et al.*, 1967) and appears to be the case in cattle as there has been found to be a direct positive linear correlation between calcium level and calcitonin level (Care *et al.*, 1970). A basal level of secretion exists even when hypocalcaemia is present (Klein and Talmage, 1968).

The circulating serum calcium level is not the only stimulus for calcitonin secretion. There appear to be other calcitonin secretagogues such as gastrin (Luthman and Persson, 1977) and the gastrin analogue pentagastrin (Luthman & Persson, 1975). Both apparently stimulate calcitonin secretion and lead to a fall in the level of serum inorganic phosphate as well as calcium. Glucagon (Care *et al.*, 1970) has also been suggested as stimulating calcitonin secretion; theophyllin is thought to produce its hypocalcaemic effect in sheep when administered at a low infusion rate by stimulating calcitonin output (Persson and Luthman, 1975); and insulin administration, which causes a fall in blood calcium in intact sheep but not in thyroidectomised sheep, is believed to achieve this through calcitonin release blocking resorption of calcium from bone (Persson and Luthman, 1974b).

There also appears to be an age response to calcitonin. In most animals the younger animal is the more sensitive (Care and Duncan, 1967) and in the trials with theophyllin (Persson and Luthman, 1975a) young sheep showed a greater response to theophyllin infusion than the older stock. The reverse appears to be the case with cattle where it has been found that young animals are less responsive than older animals (Barlet, 1968a, b). Successive treatments with calcitonin injections tend to result in a refactory

state, i.e. calcitonin no longer has a hypocalcaemic effect (Barlet, 1968a).

There has been found to be a factor present in the serum of cattle which depresses serum calcium level and this effect is greatest in the periparturient period (Barlet, 1969); calcitonin may be that factor since exogenous calcitonin depresses serum calcium one month after parturition regardless of previous hypocalcaemia history (Barlet, 1968b) and cows whose calcium level fell low enough developed a clinical syndrome indistinguishable from milk fever (Barlet *et al.*, 1971). Furthermore calcitonin levels are higher in cattle which develop post-parturient hypocalcaemia than those which do not (Black and Capen, 1973). Even in paretic cows with high circulating parathyroid hormone levels, the calcitonin level is still elevated (Garel and Barlet, 1975). Secretion of calcitonin could also explain the apparent increase in incidence of parturient paresis following an oversufficiency of calcium in the diet during the non-lactating period. In some cases of milk fever however, there is neither evidence of increased levels of calcitonin in the peri-parturient period nor evidence of secretion on ultra-structural examination of the thyroid gland (Mayer, 1970). It seems evident that parturient hypocalcaemia has a multifactorial etiology in which calcitonin may play a part - much yet remains to be learned about this condition.

Oestrogen is another hormone which has a direct effect on ultra-filtrable calcium levels (Bach & Messervy, 1969) an action which could explain why hypocalcaemia does occasionally occur following oestrus. Muir *et al.* (1972) have demonstrated that the major involvement of high blood oestrogen in the induction of parturient hypocalcaemia is through inhibition of bone resorption rather than through any decreased intestinal absorption caused by inappetance.

Growth hormone is also involved in calcium metabolism and exogenous growth hormone increases the rate of calcium absorption, calcium accretion to bone, resorption of calcium

from bone and the skeletal retention of calcium (Braithwaite, 1975a).

A number of physiological and other factors influence serum calcium levels including: -

a) Season

A seasonal change has been recorded (Shirley *et al.*, 1967) and quantified as a fall in spring to 9.0mg/100ml from 10.0mg/100ml in the winter (Claypool, 1976). Ambient temperature apparently has no effect on serum calcium (Brody, 1949) though it has been claimed that the serum calcium level falls in acute cold (Sykes *et al.*, 1969). Ross and Halliday (1976) state that season has little effect on serum calcium levels.

b) Pregnancy

Pregnancy has been claimed to have no consistent effect (Payne and Leech, 1964; Lane *et al.*, 1968) yet milk fever prone cows tended to be in a negative calcium balance towards the end of pregnancy when compared with those cows which were not milk fever prone (Manston and Payne, 1964) and a fall in serum calcium has been reported in all cows in the last month of pregnancy (Rowlands *et al.*, 1975). There does appear to be some limit to the transfer of calcium across the placenta to prevent potentially fatal demands by the foetus (Twardock *et al.*, 1971); this is important in the cow where there is no reverse flow of calcium from the foetus to the dam (Symonds *et al.*, 1966) as occurs in sheep and other animals (Symonds *et al.*, 1972).

c) Parturition

There is a fall in serum calcium pre-partum which appears to start gradually one or two weeks before parturition (Anderson *et al.*, 1970); there is a faster rate of fall in the last one to two days with calcium levels reaching the lowest point twenty-four hours post-partum (Moodie *et al.*, 1955). Other workers claim there is little pre-partum effect but in the forty-eight hours post-partum

there is an average 12% drop in serum calcium (Belyea *et al.*, 1975; Verdaris and Evans, 1976). Kirchner *et al.* (1977) similarly reported a fall after parturition but claimed that the calcium level did not return to normal for a further seven days. It has also been stated that there is a fall in foetal serum calcium in the pre-partum period, that this is larger than any maternal fall, and in both foetus and dam ionised and total calcium fall to the same degree (Wilson *et al.*, 1977).

The association between fatty tissues and calcium has also been studied at parturition. There has been reported to be an increased amount of fat mobilization around parturition (Flatlandsmo, 1971) and in sheep, as lipolysis advanced, calcium fell proportional to the rise in NEFA in the plasma. There was also a measureable increase in adipose tissue calcium (Mosely and Axford, 1971). It was suggested that the 10% reduction in serum calcium which occurred forty to sixty minutes after stress was applied, resulted from calcium moving out into fat deposits (Mosely and Axford, 1971) and parturition could be regarded as a stressful event.

d) Lactation

In a trial in Oregon, Claypool (1976) found that for the first 100 days of lactation the average calcium level was 9.41 mg/100ml, from 100-200 days of lactation 9.70 mg/100 ml, and over 200 days of lactation 9.96 mg/100ml. It is possible that the state of calcium reserves and previous dietary history could influence this (Belyea *et al.*, 1976). Certainly any changes reported do seem to be greatest over the first 100 days of lactation (Rowlands *et al.*, 1975) and constitute a fall relative to pre-partum levels with a rise in the later stages of lactation.

The extent of the lactation change may be modified by seasonal effects (Hewett, 1974). In Hewett's profile for example it was found that lactation had an effect on serum calcium level in the spring but not in the autumn.

A positive correlation between calcium values and milk yield has been found (Saarinen, 1950) with serum calcium level falling with increasing milk yield (Payne *et al.*, 1974). In cows subjected to periods of alternating milking and non-milking, hypocalcaemia followed the initiation of each milking, inorganic phosphate level was down and serum magnesium level was elevated. Non-milking was found to induce a temporary hypercalcaemia. Often there was found to be a three day time lag from the initiation of milking to the development of the hypocalcaemia, equivalent possibly to the interval between calving and milk fever in the natural state (Littledike, 1976).

e) Effects of Feeding

A number of the hormones developed in the monogastric animal for the control of post-prandial hypercalcaemia are still active in the bovine and with the continuous spillover of ingesta from the rumen to the abomasum it might be thought possible that there is little or no post-prandial hypercalcaemia.

A rise in the serum calcium level and in calcium excretion has been reported to occur after feeding though not to a marked degree (Stacy, 1969; Coggins and Field, 1976).

f) Genetic Influences

A genetic component, measured by the constancy in serum calcium levels that exists between members of a set of identical twins (Anon., 1949; Wiener and Field, 1971; Care *et al.*, 1970), has been calculated to have a heritability of 0.39 (Simon *et al.*, 1978). Breed differences which indicate a measure of genetic control also exist. Ayrshires have been found to have lower values than Ayrshire x Friesian crosses which in turn were lower than Friesians (Kitchenham and Rowlands, 1976a; Rowlands *et al.*, 1977). The level of serum

calcium is more constant for individual sire groupings of cattle (Kitchenham and Rowlands, 1976) and there is also a breed susceptibility to parturient hypocalcaemia (Hibbs *et al.*, 1946; Curtis *et al.*, 1970).

g) Age

Serum calcium falls with increasing age, with most of the change taking place prior to maturity but nevertheless continuing with increasing years as the bone becomes less soluble (Hansard *et al.*, 1957; Payne and Leech, 1964; Shirley *et al.*, 1967; Tumbleson *et al.*, 1973b; Dishington, 1974; Hewett, 1974; Bide and Tumbleson, 1976). This explains the increase in incidence of milk fever with age (Dishington, 1964) that is seen in the field. Whether the effect is more marked in cattle is not known but it has been reported in sheep that there is little change in serum calcium level due to age (Healy and Falk, 1974).

h) Diurnal Change

Coggins and Field (1976) did report a diurnal change in serum calcium level but this could have been related to feeding, a criticism that could be directed at other reports suggesting diurnal rhythms exist (Unshelm and Rappen, 1968; Unshelm and Hagmeister, 1971). The maximum serum calcium level was recorded between 1000 and 1200 hours (fed 0600 hours and milked just prior to that); thus it could be a post-prandial fall in response to the hormone to prevent hypercalcaemia followed by a later recovery. There was an interaction between cows and days in the trial and the between hours response accounted for only 5% of the variation.

The best known effect of a fall in serum calcium is parturient paresis. Once the serum level falls below 8.0mg/100ml there are changes in body function with paresis generally occurring when the serum levels fall to below 5.0mg/100ml; as the

condition progresses further the animal may eventually die (Kronfeld and Ramberg, 1970).

The primary cause of the paresis is the depression of neuromuscular transmission (Bowen *et al.*, 1970) and this applies to smooth muscle as well as striated, so there is a fall in gut motility which is worst in the more severely affected cases (Moodie, 1960). Not every case however proceeds to the extent of causing paresis, some fall to a moderate degree and recover from that point (Mayer, 1966b, Nurmio *et al.*, 1974). The serum level of calcium appears to be the critical factor as it has been found that the calcium content of the muscle from paretic and non-paretic cows is not significantly different (Kowalczyk and Mayer, 1972).

In addition to hypocalcaemia, affected cows frequently develop lower levels of serum phosphate and higher levels of serum glucose than non-paretic cows (Luthman and Persson, 1975). The latter may be due to hypo-insulinism which results especially if the case is prolonged (Littledike *et al.*, 1968). Increased fat mobilization to provide energy needs could be expected to occur and since lipolysis is associated with the uptake of calcium by adipose tissue, this would tend to lower serum calcium still further (Mosely and Axford, 1971).

Hypocalcaemia may also be associated with other disorders such as grass staggers (Hemingway *et al.*, 1965) and prolapse of the uterus in sheep (Stubbings, 1971). The calcium level has also been found to be lower from birth in those calves which later developed a scour (Cabello and Michell, 1977). A longer term deficiency may cause depressed food intake, reduced weight gain and/or reduced milk production (Underwood, 1966). Osteomalacia has been reported in animals suffering from a chronic deficiency (Church, 1971). Finally, as a result of the use of a profile in beef cattle for measuring calcium, phosphate and magnesium, it was found that cows with subnormal values for these parameters were less

aggressive, ate less food, and suffered from losses in potential production (Church *et al.*, 1978).

The serum calcium level reported seems to vary with the particular investigation; the analytical method used probably plays some part in this. Levels quoted are 9.18 mg/100ml (Church *et al.*, 1978), 9.5mg/100ml (Payne *et al.*, 1963), 9.8mg/100ml (Larson *et al.*, 1980), 10.2mg/100ml (Mylrea and Bayfield, 1968), 10.5mg/100ml (Bogin *et al.*, 1974), and 10.7mg/100ml (Bogin *et al.*, 1976). The latter paper quotes the calcium as 45% protein-bound and 55% free or ionised. This percentage balance remains at a fairly constant level in spite of changes in total calcium; thus total calcium may be accepted as a reasonable measure of physiologically active calcium (Belonje, 1976b).

Inorganic Phosphate

This element has a number of important functions throughout the body both as an organic compound or in its inorganic state. The main depository is in the skeleton with 80-85% occurring in the bony structures and the teeth. The comparatively minor but important portion present in the body fluids is involved in vital cellular structures and serves in the synthesis and degradation of numerous carbon compounds. High energy phosphate bonds play a fundamental role in the storage, liberation and transfer of energy (Simesen, 1970). Carbohydrates such as glucose are absorbed through the mucosa as phosphorylated compounds, phospholipids are believed to be the chief means by which fatty acids are transported throughout the body, and compounds such as glucose - 6 - phosphate and triose phosphate are vital intermediates to the glycolysis pathway of intermediary metabolism. Phosphorus is also found in nucleic acids (Church, 1971). Furthermore the ability of phosphorus to be excreted either as $H_2PO_4^-$ or HPO_4^{--} gives a broad margin for the regulation of the acid/base balance in the body (Simesen, 1970).

In the body, that phosphorus not contained in bone and tissue is present mainly as organic esters of phosphorus within cells; they also contain small amounts of inorganic phosphate at any given time. Serum contains approximately 14 - 15mg/100ml of total phosphorus: 5 - 8mg/100ml of this is lipid phosphorus. A trace of the remainder is ester phosphate and the most significant portion of the balance is inorganic phosphate (Simesen, 1970). One of the problems of analysis for serum inorganic phosphate is that lysis of red cells may release considerable quantities of phosphate into the serum thus altering the result (Smith *et al.*, 1975).

Normal adult bone contains 25% ash and 17% of this is phosphorus (Simesen, 1970; Church, 1971) present largely as part of the tricalcium phosphate molecule. However the bony matrix appears able to accept various ionic groups into the

crystal lattice without changing its structure and this may be one of the ways in which bone acts as a store for a number of mineral ions. Young bone is different to adult bone with the octocalcium phosphate which governs the solubility of young bone gradually changing to hydroxyapatite in adult bone, a difference that offers an explanation for the higher serum inorganic phosphate which occurs in the young (MacGregor and Brown, 1965). However calcium and phosphorus always occur in bone in approximately a 2 : 1 ratio and this is not altered where some conditions of demineralization occur so that bone estimations of calcium : phosphorus are not a good indication of the animal's inorganic phosphorus reserve (Duncan, 1958).

An important point concerning bone phosphate is that although the calcium is readily mobilised to maintain serum calcium, the phosphate is not nearly so readily mobilised to maintain the level of serum phosphate; a low serum phosphate level may therefore be one of the first signs of a dietary phosphate insufficiency (Simesen, 1970). Others have reported similarly (L'Estrange and Axford, 1965; Church, 1971; Hewett, 1974; Reed *et al.*, 1974a, b, c; Fishwick *et al.*, 1977). The reverse also holds so that where a diet is deficient and is then supplemented, serum inorganic phosphate usually rises (Tomas *et al.*, 1967; Morrow, 1969). There have been trials in which supplementation did not result in an increase in serum inorganic phosphate (Cohen, 1973b; Noller *et al.*, 1977), although the former author (Cohen, 1974), subsequently demonstrated a significant relationship between serum phosphate and pasture phosphate - this varied with the time of day at which the sampling was performed.

The extent of deposition of mineral in bone depends on the concentration of calcium and phosphate in the serum and interstitial fluid. If the concentration of calcium and/or phosphate is too low, adequate calcification may not occur. In the young this results in rickets; in the mature animal some degree of bone resorption, especially of cancellous bone, occurs (Simesen, 1970).

A high proportion of phosphorus in the plant is bound as phytates and in monogastrics these have to be broken down completely in the stomach or a proportion of the phosphorus in the diet is unavailable to the animal. In the ruminant however, phytates are completely hydrolyzed by the rumen microflora (Reid *et al.*, 1947) so that dietary phosphates are generally completely available to these species. However, while the availability of phosphorus is high in ruminants not all that available is absorbed. The Ca : P of the diet for example has been found to influence the absorption of calcium and phosphate with the dietary level of one tending to limit the absorption of the other. As Ca : P deviates from optimum, the Vitamin D requirement also increases but the ruminant can tolerate a wider Ca : P than the non-ruminant (Wise *et al.*, 1963; Young *et al.*, 1966a, b). Absorption was found to be at a maximum when the Ca : P was 2 : 1 (Manston, 1967).

Westerlund (1956), working with milking cows, found that faecal phosphate rose with increasing calcium and decreased with increasing phosphate in the milk. Reduction of phosphate in the feed led to an increased loss of calcium. These results indicated that if reserves were withdrawn from bones, the mineral in excess would be excreted. In the case of magnesium, different supplemental levels apparently had no effect on serum inorganic phosphate (Dutton and Fontenot, 1967).

Zinc sulphate added to the diet of lambs has been reported to decrease the absorption of phosphate (Thompson *et al.*, 1959) and copper or molybdenum added to the feed of steers either decreases phosphate absorption and/or increases its rate of excretion, the latter being considered the more likely (Shirley *et al.*, 1951).

Sudden dietary changes have been shown to result in reduced blood phosphate levels with no apparent effect on the phosphate concentration in milk (L'Estrange and Axford, 1966).

High nitrogen containing forages have been indicated as causing an improved retention of phosphate and higher serum levels in cattle (Stillings *et al.*, 1964) while a reduction in the amount of protein in the diet may result in increased urinary phosphate and lowered phosphate retention in calves (Mudgal and Ray, 1967). Since energy and protein are generally in an inverse ratio in the diet, this effect of protein may really be a reflection of dietary energy as Simesen (1970) found an inverse relationship between serum phosphate and energy balance.

Differences in serum inorganic phosphate due to differing feeds may not be marked as, although Belyea *et al.* (1975) did find differences due to differing feeding regimes when the change was fairly abrupt, all values were in the normal range. Sheer quantity of food ingested may be important as it has been found that serum inorganic phosphate levels were highest in a group of high yielding cows (Hewett, 1974), a result that reflected a very vigorous feeding programme for production. Most forage for ruminants however, is little more than adequate in phosphate content (Church, 1971).

With respect to Vitamin D, Ewer (1951a, b) reported that a single massive dose of the vitamin resulted in a temporary cure of rickets and improved the phosphorus retention of sheep maintained on a low phosphorus diet. Conrad *et al.* (1956) have also shown that massive doses of Vitamin D increased the absorption of P^{32} in cattle although net retention was little influenced once excretion, mainly via the urine, increased.

Absorption appears to occur at two sites in the bovine as determined by tracer studies using P^{32} in dairy cows (Lofgreen *et al.*, 1951). These appear to be the omasum and the jejunum. The rumen is relatively impermeable to the phosphate ion (Phillipson and Storry, 1965). While the abomasum has been suggested as a site for absorption (Chandler and Cragle, 1962) the small intestine does appear to play the major role

(Chandler and Cragle, 1962; Kay and Pfeffer, 1970; Grace *et al.*, 1974) with the large intestine absorbing relatively little (Grace *et al.*, 1974). It has been reported that phosphorus absorption is a passive process ; thus during the initial stages following increased phosphorus intake Manston (1967) recorded absorption through an increased proportion of the intestine until increased secretion at higher serum levels resulted in the proportion of the bowel in which net absorption occurred being reduced.

A major problem in assessing the absorption of phosphate is the quantity of endogenous phosphate added to gut contents. This endogenous addition is both a variable amount and forms a variable percentage of the faecal phosphate content. It has been estimated to be 10 - 14 g per day (Lomba *et al.*, 1969a). Most of the phosphate in the rumen appears to come from the saliva (Shirley *et al.*, 1951; Lofgreen *et al.*, 1951; Smith, 1955a; Grace *et al.*, 1974), although some secretion from both the rumen and omasum occurs. There does appear to be an additional endogenous loss in calves arising from phosphate contained in the gut secretions (Smith, 1955a, b), a phenomenon also reported in adult cows although the actual amount appears to be small (Van T'Klooster, 1969).

One effect of this salivary phosphate secretion is the reported negative correlation between serum inorganic phosphate and rainfall (Rollinson and Bredon, 1960), a higher rainfall and wetter feed reducing saliva flow. Healy and Falk (1974) reported a minor fall in serum inorganic phosphate with water deprivation, an observation that is consistent with increased salivary flow and endogenous loss.

The control of the level of phosphate in serum appears to depend largely on the absorption from and secretion to the bowel. Clark *et al.* (1973) infused di-potassium hydrogen phosphate intravenously into sheep and most of this appeared in the faeces over the next four days. They were able to demonstrate, by the use of a marker, that most of this

phosphate was added to the gut by way of the saliva and that the level of phosphorus in the saliva was related to the level of inorganic phosphate in the serum. This confirmed the earlier work of Tomas *et al.* (1967) who proposed the cyle : rumen phosphate content - phosphate absorption - serum phosphate - salivary phosphorus; with the dietary phosphate mainly available but the salivary phosphate of variable and generally low availability, there was an irreducible endogenous loss leading to hypophosphataemia where dietary deficiency was present.

In animals other than ruminants, the major mechanism of control is excretion via the kidney under parathyroid control. There is a close inverse relationship with calcium (Irving, 1964). The parathyroid hormone controls phosphorus by depressing kidney tubular resorption and permitting a greater proportion of the phosphate to be passed out in the urine (Samiy *et al.*, 1960, 1965; Mayer *et al.*, 1966a). That the urine does not constitute a major route of phosphorus excretion in ruminants is quite important as in cases where excessive phosphorus intake does increase serum levels, and there is an increased overflow into the urine, the phosphorus compounds which have a limited solubility in alkaline urine may precipitate out causing uroliths (Crookshank *et al.*, 1967). Thus the excretion of phosphorus by the salivary route not only has the advantage of buffering rumen pH, it also reduces the risk of phosphate urolithiasis in the ruminant animal.

Nevertheless parathyroid hormone has an important role to play in the control of serum phosphate in ruminants. In a series of trials in cattle, Mayer *et al.* (1966a; 1967; 1968) demonstrated that the administration of bovine parathyroid hormone to dry cows increased urinary excretion of phosphorus with a peak at 3-5 days after treatment. It did this by increasing urinary phosphate and decreasing urinary calcium. Furthermore parathyroidectomy both decreases urinary phosphate and increases urinary calcium, and the administration of parathyroid hormone corrects this situation.

Parathyroid hormone is thought to have other effects in maintaining serum phosphate levels other than those on urinary excretion. At a physiological level this hormone is thought to increase the exit of phosphorus from cells (Talmage, 1972) with most cells responding to some extent, and it has also been suggested that parathyroid hormone can regulate the rate of removal of phosphorus from the extra-cellular fluid (Foulkes and Perry, 1959). This hormone has also been reported as causing an increased uptake of phosphorus from the bowel (Borle, 1972) although in a later experiment Mayer *et al.* (1968) did not comment whether the decrease in faecal phosphate observed under the influence of exogenous parathyroid hormone was due to mineral uptake or decreased endogenous loss.

Other hormones have similarly been reported to affect serum phosphate levels. Calcitonin causes a marked decrease in plasma inorganic phosphate levels (Kennedy and Talmage, 1972), an effect which is as great as if not greater than the calcium response and which may occur in the absence of the calcium response. Thyroidectomy has also been reported as causing increased serum inorganic phosphate levels in sheep (Inskeep and Kenny, 1968) although the parathyroid would appear to provide the more dominant hormone since when both the thyroid and parathyroid glands were removed the plasma inorganic phosphate level fell and stayed low but there was an increased variability (Payne and Channings, 1964).

An important source of phosphate for maintaining serum inorganic phosphate levels is the bone as Yarrington *et al.* (1976) administered a bone resorption inhibitor and, after calving, cattle fed a balanced diet developed hypocalcaemia and hypophosphataemia even in the presence of a responsive parathyroid gland. Plasma immuno-reactive parathyroid hormone levels were similar pre-and post- partum between treated and control animals. The calcitonin assay was similar for both groups but readily mobilizable calcium was still lower between treated and control animals at sixty

days post-partum. The existence of an active bone reserve could explain why serum inorganic phosphate was held within fairly narrow limits during a six month underfeeding trial in both cattle and sheep (Healy and Falk, 1974; Roberts *et al.*, 1978); the mechanism is so effective that reverse passage across the placenta has been recorded in sheep indicating that foetal bone phosphate is also mobilizable (Symonds *et al.*, 1966).

Further hormones which may influence serum inorganic phosphate include growth hormone, which has been found to increase blood inorganic phosphate, and oestrogen, which has been found to raise blood inorganic phosphate. Again there is a possible interaction with calcium since oestrogen also affects the calcium metabolism.

Loss of phosphorus in ruminants, as already outlined, takes place largely by the endogenous loss in the faeces, the urinary loss being quite small and under normal conditions representing only 1% of the phosphate lost in the faeces (Simesen, 1970). The remaining loss of phosphorus is in the milk, active secretion increasing the level to approximately seven times the content of that in serum although the milk content may vary quite widely (Lomba *et al.*, 1969a).

There are several factors associated with a change in the serum inorganic phosphate level: -

a) Season

In a number of reports serum inorganic phosphate showed a distinct change with the time of year reaching a peak in late autumn, reflecting levels of phosphate in the pasture (Bisschop, 1964; Shirley *et al.*, 1967; Payne *et al.*, 1974). In other herds the reverse has been reported with the highest values being recorded in winter and spring (Beeson *et al.*, 1944; Vaskov *et al.*, 1969; Claypool, 1976; Gardner *et al.*,1976). A fall in

summer has also been documented (Payne, 1972b; Payne *et al.*, 1974) and this appeared to be more serious in the higher yielding cows. The suggestion was that at this time of year the pasture contained barely adequate levels of phosphate to sustain the needs of high milk production.

Whether temperature is in some way associated with seasonal changes is by no means clear since inorganic phosphate has been observed to increase with both high temperature (Yousef and Johnson, 1965) and low temperature, including acute cold (Bailey, 1964; Sykes *et al.*, 1969).

Rainfall is another possible component of the seasonal effect. Reed *et al.* (1974a; 1974b; 1974c) found in a series of studies on cattle in Botswana that during the dry season the mean level for serum inorganic phosphate in a group of herds was 4.2mg/100ml and during the rainy season 6.1mg/100ml. Bone meal supplementation in the area did correct hypophosphataemia and brought about a growth response which was most obvious when nutrition was adequate.

b) Pregnancy, Parturition and Lactation

Pregnancy, parturition and lactation all appear to produce an effect on serum inorganic phosphate levels though not necessarily a very marked one. Beeson *et al.* (1944) reported a depression of serum inorganic phosphate due to all of these events and a number of authors record a marked fall in the peri-parturient period (Godden and Allcroft, 1932; Moodie *et al.*, 1955; Littledike *et al.*, 1969). Anderson *et al.* (1970) record a fall starting one to two weeks pre-partum and remaining till after calving, even after calcium levels have returned to normal. There may also be an association with milk fever as those animals which developed clinical hypocalcaemia suffered a greater drop in serum inorganic phosphate than those animals which did not (Anderson *et al.*, 1970). Littledike *et al.*

(1969) suggested that this could be a secondary effect and not primarily associated with the disease. Part of this fall could be due to the secretion of colostrum since the demand for phosphate on the first day post-partum causes a sudden increase in the requirement for both calcium and phosphate.

There are changes in the foetus also since foetal serum inorganic phosphate is higher than maternal levels over the last 26 days of pregnancy but falls at the time of birth (Wilson *et al.*, 1977). Rowlands *et al.* (1974) reported that neither pregnancy nor lactation influenced serum inorganic phosphate levels yet in a later paper Kitchenham and Rowlands (1976) indicate that serum inorganic phosphate levels are directly related to milk production for the first 305 days of lactation. Payne and Leech (1964), Hewett (1974), and Payne *et al.* (1974) are others who record changes in serum levels associated with lactation, changes which in general tended to reflect the input/output relationship associated with the adequacy of phosphate in the feed and the demands of milk production.

c) Genetic Influences

Cattle of the Ayrshire and Guernsey breeds had higher serum phosphate levels than Friesian and Jersey cattle (Anderson *et al.*, 1930) indicating a breed influence. A good genetic correlation has also been reported between identical twins (Anon, 1949) and Kay *et al.* (1976) noted that although serum inorganic phosphate is similar between twins and singletons there is less variation between twins than between calves from different dams. Healy and Falk (1974) found no difference due to breed however.

d) Age

There is a decrease with advancing age (Anderson *et al.*, 1930; Payne and Leech, 1964; Lane *et al.*, 1968; Mylrea

and Bayfield, 1968; Tumbleson *et al.*, 1973b; Kitchenham *et al.*, 1974, 1975; Ekman, 1975; Bide and Tumbleson, 1976; Kitchenham and Rowlands, 1976). Once past two years of age however the difference is usually very slight (Healy and Falk, 1974).

e) 'Stress' Influences

Variable and inconsistent responses have been reported in respect to 'stress'. Gartner *et al.* (1969) for example found that serum inorganic phosphate was elevated when the animal was excited or aroused whereas Kriesten (1976) found it was lowered after sale and trucking. Crookshank (1976) on the other hand found no change in response to trucking, weaning or both and Healy and Falk (1974) found little change associated with rail transport.

The circumstances that applied in these investigations tended to be so different from each other that it is not really surprising that the animals responded in diverse ways.

f) Diurnal Changes

Unshelm and Rappen (1968) could not detect significant day to day changes but they did find a cow x day interaction suggesting that individual animals behaved differently on different days. Hourly variation was also significant but contributed to only 5% of the total variance involved. Cohen (1973a,b) in his study found changes in serum levels with time of day and in a later paper (Cohen, 1974) also reported a cow x day interaction.

g) Method of Rearing

Healy and Falk (1974) reported little difference in serum inorganic phosphate due to the way sheep were reared whereas Kitchenham *et al.* (1975b) found that serum inorganic phosphate was higher in rapidly reared calves and that under conventional rearing systems growth rate

was related to serum inorganic phosphate level. Little *et al.* (1977) also found a significant correlation between weight gain, feed intake and serum inorganic phosphate levels, but when weight gain was corrected for feed intake, the significance of the relationship fell.

h) Blood Sampling Technique

Teleni *et al.* (1976) reported a difference in plasma inorganic phosphate levels when samples were collected from different sites, e.g. coccygeal and jugular veins. He also found alteration in levels following the use of xylazine to quieten the animals.

An alternate method of assessing body phosphate status that has been described is by the use of bone biopsy (Little and Minson, 1977). Using this technique these authors compared the effect of different intakes of phosphate on bone phosphate content obtained by the biopsy of the last three ribs. When the comparisons were made on biopsy samples taken from the same rib, repeatability was good and the comparison valid, when different ribs were used they were not. This suggests that bone phosphate metabolism varies between sites.

Hypophosphataemia is generally found only in cases of kidney damage if dietary intake is adequate. In view of the decreasing solubility of bone, however, hypophosphataemia may show up in the presence of a dietary deficiency, especially in the older animal.

There are several effects documented which result from hypophosphataemia : -

i) Bone effects: This is the classical work described in Africa but also described in Australia (Blood and Henderson, 1968). Inadequate phosphate and nutrition result in osteoporosis which results in the sudden onset of lameness. The condition has also been described

associated with unbalanced dietary supplementation (McTaggart, 1959).

ii) Post parturient haemoglobinuria: Madsen and Nielsen (1939; 1940) described an association between this condition and hypophosphataemia. More recent work (Martinovich and Woodhouse, 1971) has demonstrated that the relationship is not consistent and the association is probably not significant.

iii) Fertility: There are many reports of reduced fertility associated with cases of hypophosphataemia (Hignett, 1950; Hignett and Hignett, 1952; Morrow, 1969; Hewett, 1974; Reed *et al.*, 1974b; Little, 1975; Fishwick *et al.*, 1977; Hunter, 1977). The effect appears to be manifest by increased calving to first oestrous intervals (Little, 1975), by more services per conception (Hewett, 1974) and by a lower ninety day non-return rate (Hunter, 1977). The picture is not clear however as Hignett and Hignett (1952) commented that Vitamin D may also have been deficient and Teleni *et al.* (1976) reported no response to phosphate supplementation of the diet. No effect on reproductive performance was reported by Gitter *et al.* (1975) even though a proportion of the stock had severe hypophosphataemia.

iv) Milk production: It has been reported that high milk production lowers serum inorganic phosphate (see earlier discussion p 117). Hypophosphataemia also results in a lower milk production (Saarinen, 1950; Church, 1971). As cattle with lower serum phosphate levels tend to be less aggressive, this could be a result of reduced intake; conversely Fishwick *et al.* (1977) found that both intake of straw and its digestibility were reduced on a phosphate deficient diet indicating that less nutrients available to the animal could produce the reduction in milk output.

v) Growth rate: A positive correlation between growth rate and serum inorganic phosphate has been reported (Rowlands *et al.*, 1974; Reed *et al.*, 1974). Teleni *et al.* (1976) however reported no change in liveweight gain following phosphate supplementation of deficient calves.

It appears therefore that blood inorganic phosphate levels may reveal a hypophosphataemia in the lactating dairy cow without clinical signs of a deficiency being present. Hewett (1974) suggests that while serum phosphate values do not provide an exact measure of phosphate intake, a marked deviation of the herd mean indicates an incorrect dietary regime. Sykes and Field (1974) on the other hand believe plasma measurement is of no value for monitoring the phosphate status of the animal and Smyth (1976) claims inorganic phosphate has no real value in a metabolic profile. Payne *et al.* (1974) did consider omitting the measurement from the profile as at that time it was the only parameter where no herd mean had fallen outside the mean and two standard deviation baseline accepted as the measure of normality.

Some of this lack of agreement between workers probably results from differences between trials. It is apparent that a greater understanding of the metabolism of this ion is required before authoritative statements may be made on the validity of blood test results.

The following serum inorganic phosphate levels have been reported for cattle: 6.3mg/100ml (Bogin *et al.*, 1974), 4.96mg/100ml (Bogin *et al.*, 1976), 4.88mg/100ml (Coggins and Field, 1976), 5.91mg/100ml (Kitchenham *et al.*, 1976) and 8.48 - 9.4mg/100ml for calves (Kitchenham *et al.*, 1975).

CHAPTER III

MATERIALS & METHODS

Cattle from the three dairy units at Massey University were selected for this project since they reflected a number of different management systems, they were variable in breed, identical twins were available, and co-operation from managers of the different herds in enabling the work to proceed was never in doubt.

The overall project was divided into several parts with different goals: -

PART A: To establish local normal values and the effects of age, stage of lactation and season of year.

PART B: To compare local normal values obtained in Part A with those from cattle obtained in another location in New Zealand.

PART C: To examine the genetic, daily, monthly and individual animal components of the variation in values of the different parameters.

PART D: To study the influence of feeding and milking on the different parameters and determine any circadian changes.

PART A

Cattle were used from all three dairy units.

No. 1 Dairy Unit: This was a predominantly Friesian herd on town milk supply for Palmerston North city and had to meet a minimum daily milk quota. The herd was managed so that there were two calving periods, one in the spring and one in the autumn. At the time of the project the calving seasons were late July to late October for the spring calving group and early April to late June for the autumn calving group.

The milking stock grazed paddocks on an area of flat ground along the banks of the Manawatu River which had been subjected to some flooding in the past. There were two predominant soil types that the stock had been grazing over. These were river flat soils of the Rangitikei series and Manawatu series.

Soils of the Rangitikei series are subjected to frequent flooding and are classed as weakly leached, rapidly accumulating soils. Generally sandy or silt loam in texture they have little profile development, the sub soil differing little from fresh alluvium. Because of the low organic matter content these soils have only weakly developed structures and tend to compact when heavily stocked. The Manawatu series are generally restricted to slightly higher broader levees. Flooding is less frequent and because of work activity causing mixing there is less evidence of flood layering in the profiles. There is a darker silt or sandy loam topsoil with a free draining sand or even gravel sub soil often not clearly demarcated (Cowie, 1978).

Most of the paddocks were established stands of a perennial rye grass/white clover combination although for short periods the stock grazed pure swards of other species e.g. Tama annual ryegrass raised as a short term crop. The paddocks were irrigated with spray irrigation for that portion of the year that rainfall was inadequate.

During the time of the investigation (July, 1972 - June, 1973) dry stock were away from the farm and grazed on the hills fronting the river. These hills consist of a series of flats and valleys. The flats on top are of the Tokomaru series as will be described in detail for the No 3 Dairy Unit. The valleys formed as a result of water erosion have valley sides and scarps of Halcombe hill soils. This is a yellow grey earth, generally a mixture of silt clay and stone with many brown mottles (Cowie, 1978). The pasture species grown on this area were of a similar type to those where the milking herd was grazed on the river flats. Approximately three weeks before they were due to calve the cows were brought down to the river flats to aid management over the calving period.

Sixty three of the 132 milking cows available were selected for this stage of the project; all were of the Friesian breed. Approximately equal numbers were taken from each of the autumn and spring calving groups. The group was otherwise selected to maximise the ranges of calving dates, ages and production records.

Cattle were sampled at four weekly intervals commencing 3 August, 1972 and continuing until 5 July, 1973. Animals lost to the trial once it had commenced were not replaced. Seventeen animals were lost by deaths and culling during the course of the investigation but in only three cases did this affect more than the last two or three samples.

This unit has been referred to in the text as Unit 1.

No 2 Dairy Unit: This was a mixed group of animals made up entirely of identical twins purchased as neonates from the southern half of the North Island. Calving for this herd was seasonal and confined to the period from early July to late September. In as many cases as possible pure Friesian or pure Jersey breeds of cattle were selected, and in all cases, both members of a set of twins were sampled. Otherwise the same selection criteria were used in this herd as were used in the case of Unit 1. A total of 34 animals from a herd of 62 were selected to take part in the trial.

For the period from commencement of the trial on 20 July, 1972 until April, 1973 these animals were grazed over the same river flats as the cattle from Unit 1. Two animals not both members of the same twin pair were lost with bloat early in the trial and one of the surviving twin members was culled for low production later in the season leaving 31 animals from the initial 34 selected. These, together with the balance of the herd were then transferred in April 1973 to a new area described in the next section as the No 3 Dairy Unit; they have remained there since. Blood sampling commenced 20 July, 1972 and was continued for 13 four weekly samples.

This unit has been referred to in the text as Unit 2.

No 3 Dairy Unit: This herd was an all Jersey herd, constituting a seasonal factory supply unit. At the time of the trial calving took place between early July and late September. During Part A of the project those animals which had not calved by mid September were injected with 20 mg Dexamethasone trimethylacetate intramuscularly to induce premature calving.

The herd of 112 animals was at the time run as four separate small herds under the one manager. The reason for this was that the cattle were involved in an experiment examining the effect of two different stocking rates and two differing levels of application of nitrogen for each stocking rate on milk production per annum and per acre. Fifty seven animals were selected from the 112 available to give as wide a range as possible of calving dates, ages and past production performance. Approximately equal numbers were selected from each of the four smaller herds on the unit.

The No 3 Unit herd was grazing an area away from the river flats, yet in close proximity to the University. The soil type was one of the Tokomaru series, a yellow grey earth. The top soil is a silt loam, the B horizon is a compact clay with many abundant brown mottles. At about 75 cm this soil type has a compact Fragipan with vertical grey graining. This Fragipan results in poor drainage and a perched shifting water table. It rises causing a wet soil in the winter with reducing conditions and a high ferrous ion concentration, and in summer the water dries out extensively to give a dry top soil with oxidising conditions (Cowie, 1978). Drainage has been laid to overcome the problems of excessive water causing pugging during high rainfall periods and during the dry time of year supplementary feeding with silage and hay was undertaken. The hay and silage fed out were usually prepared on the property from the excess of grass available during the peak growth period. At the time of this trial silage was fed at 5 kg dry

matter per head per day from 11 February, 1973 to 10 March, 1973. From then until drying off cattle were fed hay at the same rate. Blood sampling commenced on 13 July, 1972 and was carried out on 13 occasions at four weekly intervals.

This unit has been referred to in the text as Unit 3.

Collection of Blood Samples

To cancel out any differences due to diurnal variation and to fit in with the farm labour as much as possible, a standard procedure was adopted. Animals being milked were held back in a small pen adjacent to the cow shed after the morning milking had been completed and dry stock which were to be sampled were also brought in from their paddocks at this same time. On the occasions when stock at Unit 1 had to be brought from the run-off, this was carried out on the previous afternoon and the cattle were left to settle overnight close to the cow shed. The bleeding was carried out at the same time on each occasion starting at 9.15 a.m.

The cattle were bled at Unit 1 with nose grips in place and the head held round to the right after they had been driven into a crush and secured with a head stall. The sample was invariably taken from the jugular vein. Although these cattle were handled quietly, their previous experience in this bail and head stall made a number of them apprehensive; on occasions the use of considerable persuasion was necessary to induce some of them to enter the bail. These problems became noticeably less evident as the trial progressed.

On Unit 2 the cattle were milked through the same shed as Unit 1 cattle, so the same bail and head stall were used. As this particular group had not previously been used for the same range of teaching and experimental procedures, they were easily handled. However, when they were shifted in April 1973 to the Unit 3 milking shed and area, bleeding was performed in the manner described for that Unit and quiet handling became

impossible. They were now being milked in a walk-through shed whereas they had been used to a herring-bone shed. As a consequence they could only be persuaded to enter the bails with considerable shouting and occasionally other forms of inducement. By the completion of this part of the trial they had become familiar with the shed and handled more easily.

The milking shed at Unit 3 was a walk through type and the bleeding was carried out in the milking bails. The heads were secured with nose grips and pulled round to tense the jugular vein. Those cattle familiar with this unit appeared to accept the procedure with a minimum of fuss.

The technique of bleeding was kept as uniform as was possible. The jugular vein was raised with the thumb and the area of skin over the vein swabbed with a large piece of cotton wool soaked in methylated spirits. Collection was in standard evacuated blood collecting tubes. Two tubes were collected for each sample from Unit 2 and three tubes from cows from Units 1 and 3.

The first tube collected was a plain tube (Venoject[1] 15 ml) which was silicone coated and used for serum samples. Second tubes contained the additive fluoride/oxalate (F/O); these were used for haematological examinations and for plasma glucose/urea nitrogen estimations. In the initial stages Vacutainer[2] 10 ml tubes coded 3,200 PS were used; in the second half of the trial Venoject[1] 10 ml tubes coded T200 PS were used. Both brands contained the same amount of additive (20mg potassium oxalate and 25 mg sodium fluoride) and when 20 samples were bled into each of these two brands at the same sampling and tested no between tube difference in results was detected. Any third samples were taken into heparinised tubes and used for plasma alpha-mannosidase estimations. The heparinised tubes used for this were always Vacutainer tubes[2]

1. Jintan Terumo Co. Ltd., Tokyo, Japan
2. Becton Dickinson & Co., Rutherford, N.J., U.S.A.

either 7 ml or 10 ml with sufficient heparin added to inhibit clotting for 72 hours.

Tubes were fitted into a standard Venoject holder into which had been screwed a 20 guage 25 mm long thin walled Venoject[1] blood collecting needle. Animals were bled either singly as described for the Unit 1 facility or in batches in the Unit 3 facility. In each case a fresh needle was used for each cow. The bleeding was completed as quickly as possible, the samples returned to the laboratory, sorted into numerical order and the numbers from the bleed recorded to give the sequence in which sample analyses were performed.

PART B

Help was obtained from the Senior Veterinarian attached to the Rangitaiki Plains Dairy Company at Edgecumbe in the Bay of Plenty. The Dairy Company owned and managed a farm immediately adjoining the Dairy Factory and was planning to milk 250 cows in the 1973/74 season. The farm had been recently acquired and the ages and production performances of most of the cattle were not known. They were of mixed dairy breeds and included a number of crossbred animals. The animals selected were identified by reference to a chart of random numbers. Twenty animals were selected between the numbers 1 and 100, twenty animals between 101 and 200, and ten animals between 201 and 250. The 50 cows were bled once monthly, mainly at four weekly intervals, but occasionally at five weekly intervals for 1 year. The haematocrit measurement was carried out at the veterinary laboratory attached to the Dairy Company and the serum and plasma samples centrifuged and transferred to small capped plastic Auto-Analyzer cups[2] which were clearly identified and then frozen. These were despatched to Massey University by air on a timetable calculated to give minimum delay. The samples were collected

1. Jintan Terumo Co. Ltd., Tokyo, Japan
2. Technicon Corporn., Tarrytown, N.Y., U.S.A.

from Palmerston North Airport four and one half hours after despatch and immediately placed in a deep freeze. It was noted that a few samples had thawed to the point of having some liquid in them but most were still frozen solid. Generally the samples were trans-shipped in batches of about three months collections. The sampling was carried out on this Unit from July 1973 to June 1974 and the analyses were carried out over a 12 month period.

This unit is referred to as the Awaroa herd.

PART C

Sets of twins running on Unit 3 were selected for study. Initially eight sets (7 sets of 2 year old twins and one set of 3 year old twins) were selected but due to losses some replacement twins were obtained. Thus over the fifteen month period from April 1975 to July 1976 only five sets were bled throughout but seven sets were bled for thirteen months. The twins were mixed breeds consisting of Ayrshire, Friesian, Jersey and some crossbred animals. Bleeding took place on the first Monday, Tuesday and Wednesday of each month.

For this stage of the project these twin sets were bled in a head-stall and bail which had been constructed at the unit. This procedure was adopted since they were young animals, not familiar with the milking bails, and it was considered inadvisable for their first contact with the milking bails that they should be exposed to venepuncture. Most of the twin sets rapidly became familiar with the bleeding procedure and appeared to show little resentment to it. They were led into the bail, their head secured with a head stall and the head pulled round to either the right or left side. Since some had rings inserted into their noses to prevent suckling of their twin, securing this generally provided adequate restraint of the head. If no ring was present nosegrips were inserted.

Venepuncture was carried out by the method described earlier. One twin pair however, Nos. 139 and 140, continued to show a marked reaction to the application of the nosegrips. They were the only pair to show this relatively consistent reaction. Unless rapid steps were taken to prevent it the animal twisted around and fell to the ground as soon as the nosegrips were applied. Thus for nearly half the samples from this pair, venepuncture was performed on one or other twin in dorsal recumbency. The samples were handled in a similar manner to those described for Part A of the project. Although these twin sets were taking part in milking machine trials they were grazing pasture as one group and being milked twice a day. It seemed unlikely that the trials with which they were involved would cause alterations in blood chemistry.

PART D

Cattle:

For this stage of the project twelve animals were selected from Unit 3, i.e. 6 twin pairs. In this instance they were mature mixed-aged Jersey, Friesian, or crossbred cattle. The cows selected were all due to calve very early in the calving season and any not calved 18 days prior to the onset of the project were induced to calve prematurely by injecting them intramuscularly with 20 mg Dexamethasone trimethylacetate. As a consequence some of the animals retained their foetal membranes and developed a low grade metritis. There were no systemic signs of illness, temperatures remained within the normal range and there was no change in the haemogram; the foetal membranes had all been voided by the time sampling began.

Prior to the start of the project the cattle were brought in and stood on a hard standing for some hours per day to strengthen their legs for the period in the barn. During the

time they were in the barn and while on the hard standing, they were fed a mixture of dried grass and dried lucerne.

Housing, Feeding, Milking & Management:

During this investigation the cattle were housed for the whole time in stanchions securing the head and leaving the animal free to stand or lie as preferred. Water was available in front of the animals all the time and feed was offered at 9 hour intervals regardless of the time of day. The feed was a mixture of dried grass and dried lucerne in equal parts. A weighed quantity was offered and at the end of 60 minutes the unconsumed portion was removed, weighed and returned to the bulk stock.

The milking process was carried out every 11 hours regardless of the hour of day or night and was done while the cattle were standing in the stalls in the barn. The milk from each cow was individually weighed and then emptied into the farm vat for daily removal. After milking the barn was thoroughly cleaned by physical removal of the faeces followed by washing with a high pressure hose.

Insertion of Catheters:

Commencing one week before the trial was due to begin the animals had indwelling catheters inserted. These were Intramedicut catheters[1] of a vinyl plastic 105 cm long. For insertion the animal was sedated with an appropriate dose of Rompun[2] given intramuscularly. When recumbent, an area over the jugular vein and another behind the top of the shoulder blade were clipped and sterilised. Incisions were made and a sterile nylon cannula with a stilette through it for rigidity was manipulated from the incision behind the top of the shoulder blade across the scapula and down to the jugular

1. Sherwood Medical Industries Inc., St Louis, Ms. U.S.A.
2. Bayer, A.G. Leverkeusen, Germany

groove. The stilette was withdrawn to provide a subcutaneous channel and the catheter was inserted through the cannula. The cannula was then removed leaving the catheter embedded subcutaneously with the capped Luer fitting through the incision behind the shoulder and the balance, held by an assistant, emerging from the incision just exterior to the jugular vein.

The method of insertion of the catheter through the vein wall was by a similar technique. A large bore needle with a plastic sleeve on it was inserted into the vein. The needle was withdrawn and the catheter inserted through the sleeve which was then slid back along the catheter. Approximately 15-20 cm of catheter were inserted into the vein before the sleeve was withdrawn and a reasonable seal was formed by the vein wall pressing on the catheter. The sleeve was slid back along the catheter as far as possible and the wound closed by the insertion of 0.6 mm Supramid sutures[1].

The catheter was then tested by aspirating blood. The tube was flushed with normal saline and left filled with a saline solution to which heparin[2] had been added to prevent clotting at a concentration of 50 IU heparin/ml of normal saline. The process was repeated for the opposite side and the cattle propped in sternal recumbency to recover. The insertion of the catheters was uneventful and normal recovery followed except in the case of one animal which developed a nerve paralysis in the right front leg. This animal was left outside on paddock grazing during the course of the trial and was offered hay *ad libitum*.

1. Braun, B. Melsungen, A.G. West Germany
2. Evans Medical Supplies Ltd., Liverpool & London

Collection of Samples:

Blood sampling was carried out at 2 hourly intervals for the first 6 days and then 4 hourly intervals for the second 6 days; sampling was for the whole of the 24 hour period per day. The cattle after the first few occasions were bled in the same order and the procedure followed was the same on each occasion. The heparinised saline in the catheter was aspirated and discarded usually with an equal volume of blood. The blood sample was then withdrawn into a 20 ml syringe, the catheter immediately flushed with sterile normal saline, then refilled with saline containing the heparin, and finally sealed with the cap. The syringe containing the blood sample was used to discharge 10 ml of whole blood into the plain tube and 10 ml into the fluoride/oxalate tube which was then inverted and re-inverted several times to thoroughly mix the anticoagulant. All tubes were identified and placed in racks.

At the end of the twelve days sampling the cattle were turned out to graze with the rest of the herd and the catheters removed over the next four days.

THE ANALYTICAL PROCEDURES

Standard procedures were adopted and kept as constant as possible for each part of the project. Because of other work commitments the methods by which samples were handled had to be modified between each part.

The following steps were undertaken for Part A of the project. In the laboratory the sample tubes were placed upright in racks. The F/O tubes were placed in a blood cell suspension mixer[1] in sequence, removed after thorough mixing and unstoppered. A sample was removed directly into a capillary tube for

1. Matburn Ltd, London, England

haematocrit measurement and a further sample aspirated by Pasteur pipette and placed in a clean 2 ml auto-analyzer plastic cup for haemoglobin estimation (see section on haemoglobin estimation). These two measurements were performed immediately. Another volume of mixed whole blood was taken when required for the manual haemoglobin estimation. The tubes were then centrifuged in a BTL bench centrifuge[1] at 2,500 rpm for 15 minutes. Plasma was then aspirated by Pasteur pipette and placed in a clean washed and dried auto-analyzer cup for the analysis of plasma glucose and urea nitrogen.

The above procedures, including the plasma glucose analyses on these samples were always completed on the same day that the sample was collected.

The serum sample was allowed to remain at room temperature for 24 hours for clot development and retraction. On the next morning the clot was removed and the serum decanted into a glass centrifuge tube and centrifuged in the BTL bench centrifuge at 4,500 rpm for 20 minutes to ensure complete sedimentation of all red cells. The serum was then aspirated (6 ml if possible, but less if clot retraction had not been complete) with a Pasteur pipette and placed in auto-analyzer cups which were identified and capped. Two of these were frozen immediately and the third was used for the initial serum tests. The protein and albumin analyses were carried out first because of possible alteration in the test results on freezing by the formation of cryo-proteins. In the initial stages of the study this was all that was able to be completed on that day and the tests for sodium and potassium were carried out on the subsequent day; later, as sample processing became more proficient, the potassium and sodium analyses were also performed the same day. When the test was carried out on subsequent days the samples were refrigerated overnight, or frozen if required to be held for a longer period.

1. Baird Tatlock London Ltd, Chadwell Heath, Essex, England

The analyses for calcium, phosphate and magnesium were carried out in batches during the week when no sampling was performed, the sample being frozen in the interim. Thawed samples were carefully remixed because of the fractionation of the sample which took place during freezing and thawing.

With the exception of the PCV and magnesium estimations all tests were carried out on a Technicon Auto-analyzer[1] consisting of the following modules: sampler 1, proportioning pump 1, heating bath, two colorimeters, flame photometer III, and a two-pen recorder. A disadvantage with the procedure that was followed was the frequent reprogramming of the various modules of the Auto-analyzer for the different assays. After even short runs the tubing manifold had to be washed and the next manifold checked for proper function and baseline noise before the standards and test sera could be run through. This disadvantage was minimised with the longer runs used for magnesium, calcium and phosphate estimations where the larger batches resulted in more efficient use of the equipment.

The most accurate and repeatable method of measuring serum magnesium was considered to be with an atomic absorption spectrophotometer (the analytical method is described later). The specimens were removed from the deep freeze and placed in a refrigerator approximately 40 hours before the test was to be performed to allow the samples to thaw slowly to refrigerator temperature ($4^{o}C$). In most instances this was done in the late afternoon. On the next afternoon the samples were remixed by repetitive inversion. Using an automatic pipetting device (Finn Pipette FP14)[2] the diluent was measured out into plastic centrifuge tubes and with a similar device (Finn Pipette FP12)[2] the serum to be assayed was added. The samples were then thoroughly mixed and the tubes capped. The analyses were occasionally carried out that afternoon but more frequently on the following morning. When performed the next morning the samples were stored overnight in a refrigerator

1. Technicon Corporation, Tarrytown, NY, USA
2. KY Finn Pipette, Helsinki, Finland

and allowed to equilibrate with the room temperature before the assay was carried out.

The individual tests were carried out as described in the text (pp 138-143). Readings were carried out manually by drawing a curve to fit the standards on the Auto-analyzer chart reader and reading the values on the print-out. In all cases except haemoglobin, magnesium, sodium and potassium, one standard at about the physiological level was inserted during the run to check the constancy of the values. For short runs this was in the middle and at the end of the run, with longer runs this was no less frequently than one per sampler tray (i.e. 1 in 40). This could not be done in the case of haemoglobin because the samples themselves were used as standards. In the case of sodium and potassium the recommended frequency of one standard every 10 samples was followed. For the magnesium estimation a blank solution of diluent was run to check the zero and a standard in the physiological range was also analysed every 10 samples.

During Part B of the project all samples were held frozen and several bleeds were analysed at the one time. Again the samples were transferred from the freezer to a refrigerator and allowed to thaw to refrigerator temperature overnight. Next day the samples were sorted into testing order, remixed and allowed to come to room temperature. In all other ways the testing procedure was carried out in an identical fashion to that described for Part A of the project.

For Part C of the project a modified procedure was adopted. The samples were collected in the morning and brought down to the laboratory. They were permitted to stand on the bench until the afternoon when the haematocrit was performed in the manner described earlier, and a sample removed for haemoglobin estimation and refrigerated. The F/O sample was then centrifuged and the plasma aspirated and frozen. The tube for serum retrieval was left at room temperature. For the second day of sampling, the samples were again left at room temperature until the afternoon when the same work procedure

was performed on that test collection. That afternoon the sample which had been allowed to clot from the previous day was centrifuged and the serum aspirated and frozen. On day 3 of collection the steps were followed as for day 2 and in addition the haemoglobin samples collected on days 1 and 2 and held in the refrigerator for the intervening period were analysed with the day 3 sample. The samples selected for the manual haemoglobin estimation were from the day 3 collection and were based on a wide variation in the haematocrit values rather than waiting until the haemoglobin assay had been carried out. On the afternoon of day 4 the frozen plasma samples were thawed, the estimations on glucose and urea nitrogen carried out, and the final batch of samples for serum were processed and frozen. The analyses on serum were carried out in batches as time permitted.

Part D of the project necessitated changes in the procedure since the large bulk of material collected meant that only minimal work could be carried out while the project was in progress. The sample collection on each occasion took one hour; a proportion of the remaining hour between samples was spent in bulk mixing of feed and weighing out individual lots of feed before and after feeding. In the balance of the time left the haematocrit was performed and the haemoglobin samples aspirated, placed in the auto-analyzer cup and frozen. The F/O tube was then centrifuged as described earlier and the plasma sample aspirated and frozen. Adequate serum could be gathered from the plain silicone-coated 10 ml Venoject tubes that were being used if allowed to stand for 24 hours for adequate clot formation and retraction.

At the end of the project catheters were removed and the animals sampled daily and then weekly by venepuncture to check changes after returning to pasture. In view of the deaths that occurred and which are discussed later, these samples must be considered abnormal and were not included in the results.

Haematocrit

The sample for the PCV estimation was taken from the collection tube after thorough mixing. Blood was allowed to flow up a micro-haematocrit tube by capillary action when both this and the sample tube were tilted to an angle of approximately 60° from the vertical. In the initial stages of the project, blue tipped Select[1] capillary tubes were used and after supplies of these were exhausted, blue tipped Terumo[2] capillary tubes were used.

After filling, the tubes were closed with Cristaseal[3], placed in a model MU International micro-capillary centrifuge[4] and spun at 11,500 rpm (13,460g) for five minutes. Following centrifugation the centrifuge was allowed to slow to a stop and the samples were then read off as a percent PCV in an International micro-capillary reader[4].

Haemoglobin Assay

The determination was based on the conversion of haemoglobin to methaemoglobin by potassium ferricyanide and subsequent conversion to cyanmethaemoglobin by potassium cyanide. The manual procedure has been described in Standard Methods of Clinical Chemistry (Lamberg & Rothstein, 1978). In the automated procedure used in this project (Technicon method N-18a) mixed whole blood samples were aspirated at a rate of 60 per hour, the samples diluted, and the red cells haemolyzed with an air segmented stream of distilled water. Ferricyanide-cyanide reagent was then added and the stream passed through a time-delay coil for colour development. The colour was measured at 550 nm with an 8 mm tubular flow cell.

1. Propper Manufacturing Co Ltd., Long Island City, N.Y., U.S.A.
2. Jintan Terumo Co Ltd., Tokyo, Japan
3. Hawksley and Sons Ltd., Lancing, Sussex, England
4. International Equipment Co., Needham Heights, Massachusetts, u.S.A.

During Part C of the project tests were carried out on whole blood samples to determine whether there was any major change in haemoglobin level due to freezing. The variation between fresh and frozen samples was found to be within the limits of experimental error.

No standard was used with this method but each run was calibrated against a manual method. A range of samples which gave widely differing values of PCV were assayed using Drabkins solution[1] prepared in the prescribed manner from 'Drabkins Powder' and the results read off on an EEL Haemoglobinometer[2]. This machine has an internal calibration and is standardized with metal plugs which occlude a fixed proportion of incident light. For the test 4 ml of Drabkins solution and 0.2 ml of blood were mixed by inverting and reinverting several times, allowed to stand for at least four minutes and then read off as g/100 ml on the haemoglobinometer.

Total Protein Assay

The method adapted for the Auto-analyzer (Failing *et al.*, 1960) is a modification of the biuret reaction as proposed by Weichselbaum (1946). (Technicon Method N21 1-II for combined serum total protein and albumin assay). This depends on the formation in alkaline solution of a purple coloured copper complex with two or more carbamyl groups (-CO-NH-) which are joined together directly or through a single atom of carbon or nitrogen.

The sample stream is diluted with an air-segmented stream of biuret reagent, mixed, and the developed colour is measured in a colorimeter in a 15 mm tubular flow cell and a 550 nm filter. For calibrating standards bovine albumin fraction V, diluted to give 2.3, 4.6 and 7.0 g/100ml, was used initially. After this was exhausted (March 1973), Wellcomtrol I[3] was used as a standard and this was also diluted to give 2.6, 5.2 and 7.9 g/100 ml of protein.

1. Diagnostic Reagents Ltd., Thames, Oxon, England
2. Evans Electro-selenium Ltd., Halstead, England
3. Wellcome Reagents Ltd, Beckenham, England

Albumin Assay

The albumin procedure was based on the quantitative specific binding of the dye 2-(4'-hydroxybenzeneazo) benzoic acid (HABA) specifically to serum albumin. The method adapted for the Auto-analyzer (Nishi and Rhodes, 1965) is based on the work of Ness *et al.* (1965).

In the procedure the sample stream was diluted with an air-segmented stream of buffered dye and mixed. The developed colour is read in a colorimeter with a 15 mm flow cell and a 505 nm filter.

For standards, the same figures as for total protein applied for the initial period and then Wellcomtrol I was used giving 1.1., 2.3, and 3.4 g/100 ml of albumin.

Urea Nitrogen

The urea nitrogen method was a slightly modified version of the procedure described by Marsh *et al.* (1965) and involved a modification of the carbamido-diacetyl reaction as applied to the determination of urea nitrogen. The method was based on the direct reaction of urea and diacetyl-monoxime under acid conditions. The presence of the thiosemicarbazide intensified the colour of the reaction product and enabled the determination to be run without the need of concentrated acid reagents.

In the automated procedure the test was run on plasma concurrently with the glucose (Technicon method N-16b) at a rate of 40 samples per hour. The sample stream was the diluent output from the glucose after passage through the dialyzer. This stream was recirculated through the dialyzer and the recipient stream was the colour reagent (thiosemicarbazide-diacetylmonoxime combination). Sulphuric acid was then added to create the acid conditions for the reaction to proceed and after mixing it passed through the heating bath at 95°C. The colour development was then read in a colorimeter with a 15 mm tubular flow cell and a 520 nm

filter. The system was calibrated with urea nitrogen standards of 5, 10, 20, 30, 40, 50 mg/100ml of urea nitrogen.

Glucose Assay

The glucose estimation in this project was carried out on plasma aspirated after centrifugation of the F/O sample. The method used was a modification of a procedure described by Hoffman (1937) utilizing the potassium ferricyanide-potassium ferrocyanide reduction method, the yellow ferricyanide being reduced to the colourless ferrocyanide.

In the automated procedure modified from Technicon method N 16-b samples taken at 40 samples per hour were injected into an air-segmented stream of potassium cyanide. This was then passed through a dialyzer where the glucose diffused through a cuprophane membrane to a recipient stream of air-segmented alkaline ferricyanide. The process removed the plasma protein and any residual erythrocytes. The output stream of the ferricyanide-dialyzed sample was incubated at 95°C in a heating bath and then the colour loss read in a colorimeter with a 15 mm tubular flow cell and 420 nm filter.

A stock glucose solution was prepared and diluted with standard diluent to give a range of glucose standards of 20, 30, 40, 50, 60, 70 mg/100 ml of glucose.

Sodium and Potassium Assays

The levels of sodium and potassium were determined simultaneously by flame photometry in a two channel module. In the procedure Technicon method N-20d used in the project the samples taken at 60 per hour merged with an air-segmented stream of acid lithium nitrate diluent, mixed in a coil and entered the dialyzer. The dialyzed ions entered an air-segmented distilled water stream flowing through the recipient channel of the dialyzer. This stream entered a glass debubbler where the segmented air exited to waste and a solid stream entered the atomiser and propane-air flame.

Lithium was used for both sodium and potassium as an internal standard. Photo cells located in a detector assembly measured sodium, potassium and lithium emissions, and the sodium-lithium and potassium-lithium differences were charted on the recorder.

A series of combined standards were used for calibration giving a range of 100 mEq/1 to 160 mEq/1 of sodium and 2 mEq/1 to 8 mEq/1 of potassium.

Magnesium Assay

The best means of testing for magnesium in terms of precision and ease is by atomic absorption spectrophotometry (Willis, 1961) and as carried out in this project this was on a model AA-5 Varian Tectron atomic absorption spectrophotometer[1].

In this method a blank solution of strontium nitrate was prepared containing 1000 ppm strontium. The purpose of the blank was to reduce the interference by other minerals, principally calcium and phosphate Each sample was prepared by dilution of 0.2 ml sample with 5.0 ml of the strontium solution. This was then aspirated into an air-acetylene flame and percent transmission at 285.21 nm read off the mode.

Calibrating standards were prepared giving 0.0, 0.5, 1.0, 1.5 and 2.0 ppm. Sample results were multiplied by a factor of 2.6 to obtain the concentration in mg/100ml in the original sample.

Calcium

The calcium procedure was a modification of the method of Kessler and Wolfman (1964). This was modified by the use of 8-hydroxyquinoline to virtually eliminate the interference of magnesium (Gitelman, 1967). (Technicon method N 26a/I).

1. Varian Tectron Instruments Ltd., Melbourne, Australia

Calcium was determined by first mixing with 0.25N hydrochloric acid to release the protein-bound calcium. The ionized calcium was then dialyzed to a recipient stream of 0.25N hydrochloric acid. Cresolphtalein complexone containing 8-hydroxyquinoline, and base were then added to the stream after it left the dialyzer. A coloured calcium-dye complex was formed in the presence of diethylamine. The developed colour was measured in a colorimeter with a 15 mm flow cell and a 580 nm filter.

A problem developed with the calcium assay during Part B of the project. Even though the simultaneous phosphate assay was successful, repeated attempts yielded no colour in the automated calcium assay of these samples in spite of the observation that colour was produced from the same reagents in a test-tube. It was later found that the assay functioned if the concentration of diethylamine was increased.

The calcium standards used for calibration were 5.0, 7.5, 10.0, 12.5, 15.0 mg/100ml of calcium.

Inorganic Phosphate Assay

The technique (Technicon method N-26a/I) was based on the formation of phosphomolybdic acid which was then reduced by stannous chloride-hydrazine. Use of this stable reducing agent was reported by Hurst (1964) and adapted for the Auto-analyzer by Kraml (1966).

The procedure was run at 40 samples per hour simultaneously with calcium, the outlet from the calcium dialysed being recycled through a second dialyzer with a recipient stream of 0.25 N hydrochloric acid. After mixing with an acid solution of ammonium molybdate and addition of stannous chloride-hydrazine reagent, the developed colour was read in a colorimeter with a 15 mm tubular flow cell and a 660 nm filter.

Phosphate standards of 1, 3, 5, 7, and 10 mg/100ml were used for calibration.

STATISTICAL ANALYSIS

PART A:

It was necessary to reduce the large amount of data to a relatively concentrated form to facilitate display and interpretation of the results of the investigations. This was done by using a combination of statistical packages and specific computer programs implemented on an IBM 1620 Mark II computer.

The initial problem was to select the simplest equation which would give an adequate description of the values for the various parameters (Draper & Smith, 1966). As a first step, the mean and standard deviation were obtained for each sampling for each dairy unit. These means were examined graphically to determine the shape of the curve that would be required to describe monthly changes in various measurements. On empirical grounds it was decided to use a polynomial regression with up to 4 powers fitted by the method of least squares (Draper & Smith, 1966) to describe the changes in each variable for each unit. A multiple regression package[1] was used to estimate parameters of the regression equation and the curve obtained displayed, together with monthly means and standard deviations using a specifically written computer program which made use of a plotting subroutine available for the IBM 1620. The Y-axis for each curve was scaled in standard deviation units adjusted for the range of mean values over all samples and all units. This procedure allowed direct comparison of all the curves for each variable. This first series of curves, where the X-variable was numerically coded (4-52) for weekly sampling date, were supplemented by a further series of curves where the X-variable was the number of weeks since calving at each sampling for each animal in each unit. A separate series

1 Program suite BAR 3 written by E.G. Burr, University of New England, Armidale.

of curves were derived for the autumn and spring calving groups of animals on Unit 1 and a final series of curves were estimated for each unit with the age of the cow as the X-variable.

PART B:

The testing procedure for Part B was similar to that for Part A and the same statistical analyses were applied. As a result of a misunderstanding however the samples were collected by calendar months and not by lunar months; on two occasions the inter-sampling interval was therefore 5 weeks and not 4 . On the basis of graphical comparisons it was decided to treat all the data as though all the intervals were 4 weeks. This allowed a direct comparison between the Part B results and the results for the three Massey Units in Part A.

PART C:

Deaths and culling during this part of the project resulted in data being available for 7 twin pairs for 13 months or 5 twin pairs for 15 months. The curves were estimated separately for 7 twin pairs with 13 samplings and for 5 twin pairs with 15 samplings. The curves fitted to the data for 13 months have been used to illustrate the changes. Otherwise the same procedures were used as in Part A. The results from this stage of the project were analysed as a four-way, mixed-model analysis of variance (Scheffe, 1959). The main effects in these analyses were months, twin-pairs and animals nested within pairs, all of which were treated as 'fixed effects', and a second nested effect, days within months, which was treated as a 'random effect'. Numerical estimates of the component of variance attributable to each effect were obtained by standard procedures (see Appendix Table C:2). For each analysis the numerical estimates (excluding those where the mean square was less than the

residual mean square) were expressed as a percentage of the sum of the estimates for all effects (= total variance for a single observation).

PART D:

The results of the first six-day cycle were analyzed as a series of regression analyses. The independent variables were amount of feed eaten, time since feeding, time since milking and time of day. Regression coefficients were estimated for each of the dependent variables e.g. haematocrit, haemoglobin, etc., with a variety of combinations of independent variables.

Initially, analyses were completed separately for each independent variable using a polynomial regression equation. Coefficients for the linear, quadratic, cubic and quartic powers of the independent variable were fitted successively. In each analysis the process was truncated to the stage where the additional power(s) gave no significant increase in the estimate of the multiple correlation coefficient. Combined analyses were then carried out for each dependent variable with coefficients corresponding to the powers of the four independent variables selected on the basis of the results of the separate regression analyses.

These combined analyses were repeated with different sequences of fitting the independent variables in order to find the simplest form of equation which gave maximal explanation of the dependent variable (Draper & Smith, 1966). Finally the residual mean square for each analysis was adjusted by removing effects due to differences between cows and days so that the final form of the equation was examined in the light of its influence on the variation of the dependent variable with the effects of days and cows excluded.

Where the effect of an independent variable was significant a specific program on a Sord M222 Micro-computer was used to plot points on a standard deviation scale for the dependent variable. A curve joining these points was then drawn by hand.

CHAPTER IV

RESULTS AND DISCUSSION (PART A)

The results discussed in this section of the project examine herd-mean differences and temporal variations in mean values of the eleven blood parameters measured. Some of the factors which contributed to the variation in values included season, stage of lactation, age and time of calving.

The mean values for the various blood parameters measured on the three units at Massey University are shown in Table IV:1. Information from the United Kingdom report (Rowlands *et al.* 1974) is shown in the same table for comparison.

The individual results were also graphed as functions of: -

(a) time from commencement of sampling,
(b) weeks in milk, and
(c) age

for each of the three units as discussed in the section on handling of the data. The separate spring and autumn calving groups on Unit 1 were also graphed separately for:-

(a) time since commencement of sampling, and
(b) weeks in milk.

Haematocrit and Haemoglobin

Since the haematocrit percentage and haemoglobin concentration are closely related the results for both parameters have been discussed together.

The mean haematocrit values for the Massey herds were approximately 14% lower than the values reported for cattle in the U.K. although the degree of variation was reasonably similar (Table IV:1). Some of the values for the Massey units were indicative of clinical anaemia according to Schalm (1965).

TABLE IV:I

MEANS (±SD) FOR BLOOD PARAMETERS FROM MASSEY DATA AND UNITED KINGDOM DATA

Parameter measured	Unit 1			Unit 2			Unit 3			U.K.*	
	n	$\bar{x}$	SD	n	$\bar{x}$	SD	n	$\bar{x}$	SD	$\bar{x}$	SD
Haematocrit %	692	25.4	2.8	386	26.4	2.9	713	26.4	3.1	30.2	2.8
Haemoglobin g/100ml	688	9.8	1.1	356	10.6	1.3	706	10.6	1.4	12.1	1.0
Total protein g/100ml	689	9.1	1.0	378	8.5	0.8	711	7.7	0.6	7.6	0.5
Albumin g/100ml	687	3.1	0.6	374	2.9	0.5	712	3.8	0.9	3.0	0.3
Urea nitrogen mg/100ml	668	22.1	5.5	376	18.8	5.0	705	16.7	8.2	14.4	2.5
Glucose mg/100ml	666	57.8	5.9	376	59.0	6.0	707	55.6	7.6	54.0	5.4
Sodium mEq/1	682	144.4	6.2	382	146.8	5.2	706	147.6	4.6	139.7	2.1
Potassium mEq/1	690	5.3	0.6	387	5.1	0.6	696	5.3	0.8	5.0	0.4
Magnesium mg/100ml	685	2.3	0.6	383	2.0	0.4	709	2.1	0.8	2.5	0.3
Calcium mg/100ml	688	9.8	1.3	385	9.9	1.4	712	10.4	1.4	9.5	0.4
Inorganic phosphate mg/100ml	688	6.0	1.3	385	5.8	1.3	709	6.0	1.3	6.0	0.9

* United Kingdom information after Rowlands *et al.* 1974a n for these figures was estimated to be > 3,500

TABLE IV:2: PERCENTAGE OF VARIATION EXPLAINED (R^2) MASSEY UNITS

	Season			Lactation			Age		
	Unit 1	Unit 2	Unit 3	Unit 1	Unit 2	Unit 3	Unit 1	Unit 2	Unit 3
Haematocrit	14.4**	9.6	23.9*	1.8	14.2*	24.8**	2.1	19.8*	1.5
Haemoglobin	6.8	0.7	3.8	1.9	2.5	24.9**	1.9	16.5*	1.7
Total protein	50.5**	35.2**	16.8**	8.9*	31.2**	5.4*	6.3	19.8*	15.1**
Albumin	33.8**	27.8**	59.9*	6.3	26.9**	39.3**	3.9	7.0	1.0
Urea nitrogen	14.7**	19.7*	65.6**	4.7	13.5*	52.9**	8.0*	11.6	0.0
Glucose	12.1*	8.1	32.5**	1.3	5.7	19.4**	7.5*	5.9	0.0
Sodium	33.7**	13.6	14.7**	5.4	8.0	15.1**	0.2	2.5	0.0
Potassium	39.6**	20.5	15.3**	11.5*	17.6*	25.5*	0.0	11.3	0.0
Magnesium	8.0*	27.6**	0.0	0.3	27.3**	1.4	0.06	1.1	0.3
Calcium	33.8**	47.1**	8.8**	13.1**	19.8*	9.6*	0.3	2.6	0.8
Inorganic phosphate	13.0**	22.3**	26.7**	6.9	10.1	18.2**	1.6	5.8	2.4

Significance of Multiple Regression coefficient (R)

** P < 0.01

* P < 0.05

Shrinkage of the erythrocyte under the influence of the anticoagulant used (Medway and Prier, 1969) could explain, at least in part, these low values but it would not explain the differences between the means from the two sets of data as Payne *et al.* (1970b) and Rowlands *et al.* (1974) used the same anticoagulant for samples taken for their haematological estimations. These differences are referred to again later in this discussion.

A similar relationship held between the Massey data for haemoglobin levels and that in the U.K. (Table IV:1) although the variation in the U.K. data for this parameter appeared to be less.

The Massey values for haemoglobin were similar to those recorded by Schalm (1965) who cited a value of 11.0 g/100ml for normal cattle.

There are a number of factors which could explain the differences between the present data and that reported overseas: -

a) Breed:

In this instance herds on Units 1 and 2 were predominantly or exclusively Friesian and the herd on Unit 3 predominantly Jersey. Differences reported in the literature (see Review of Literature) are nearly always slight when compared with the differences between these herds (Table IV:1) and the information quoted from the U.K. It is unlikely therefore that breed effects would have accounted for the differences obtained between the two countries.

b) Climate:

Climate could be another cause of variation in haemoglobin level. Early reports (Manresa *et al.*, 1934, 1939a,b) demonstrated a slight negative correlation between temperature and haemoglobin level. Later reports claimed

however that no relationsip to temperature (Brody, 1949) or a slight positive relationship (Rusoff *et al.*, 1954) existed. There are recent reports (Whitlock *et al.*, 1974; Rowlands *et al.*, 1974) of a fall in haemoglobin and haematocrit in winter and a rise in summer. Although a seasonal periodicity is indicated by these reports this has not been shown to be related to environmental temperature.

c) Nutrition:

Differences in level of protein feeding have been shown to influence haemoglobin level (see Review of Literature). While differences in the level of nutrition between the U.K. and New Zealand almost certainly exist, the average urea nitrogen levels (which are an indicator of readily available nitrogen) of the Massey herds (Table IV:1) suggested they had a surplus of readily available protein. The blood glucose levels, if these reflect an adequacy of intake as suggested by Payne *et al.* (1973), were also higher than values obtained in the U.K. Neither protein nor energy intake differences appear to provide a satisfactory explanation for the variation in haemoglobin values found between the two countries.

d) Reproduction:

Both haemoglobin and the haematocrit have been reported to fall in late pregnancy, rise at parturition and fall in early lactation (see Literature Review). In the Massey herds, by sampling the same animals for a 12 month period, an average value for these animals for the whole time has been obtained. The structure of the U.K. project, although differing in the selection of animals and timing of sampling, should have similarly eliminated the effect of those factors on haemoglobin and haematocrit levels in that country. It seems unlikely therefore that factors associated with reproduction would explain the differences observed.

Other factors discussed in the Review of Literature as having some effect on the haematocrit and haemoglobin levels e.g. stress and circadian changes, have been rendered negligible or cancelled by the structure of the sampling programme. At this point it seems that the differences in the haemoglobin and haematocrit levels recorded between New Zealand and the U.K. cannot be explained except perhaps in terms of management influences, not yet identified, which are associated with two different systems of husbandry.

Considering Figs. IV:1-36 there is considerable similarity between the corresponding haematocrit and haemoglobin plots. This is only to be expected if the content of haemoglobin in each erythrocyte remains relatively constant (Schalm, 1965) since factors which influence one of these parameters are likely to affect the other.

Examination of the mean values for both the haematocrit and haemoglobin for each of these units indicates that they followed essentially the same pattern through the 12 month period (Figs. IV:1-4 and IV:19-22). The curve of best fit does not necessarily support this comment (see for example the shape of the curve in Fig. IV:2 compared with IV:3). Sampling which began on Unit 3 three weeks before that on Unit 1 and one week before that on Unit 2 could explain phase differences in the fitted curves. If earlier samples for these latter two herds had been obtained, and they had revealed higher values than the first values actually plotted for these two herds, the shape of the curve could have been identical to that in Unit 3.

The very large standard deviation for haemoglobin shown for the final sampling on Unit 2 (Fig. IV:20) is accounted for by the accidental loss of material before the analysis was carried out. Only three samples were salvaged and these had been selected for high, medium and low values of haemoglobin to use as standards (see Materials and Methods p 138).

From the data obtained it appears that there are two high points in the year for the haemoglobin and haematocrit values - one in summer and one in winter, with the former being more prolonged. There are at least three reasons why this should be so: -

a) Direct effect of climate

Climate could have a marked effect on cattle in New Zealand, not because of the extremes that occur *per se*, but because they spend their life in an outdoor grazing situation all year round. The earliest report of a possible effect of climate was by Clawson (1914) who reported a drop in the erythrocyte numbers from early to late summer. This would coincide with the peak and subsequent fall from December to about March in the data reported in this thesis. Other workers have tended to find a single simple curve with a high in summer and a low in winter (Rowlands *et al.*, 1974a; Whitlock *et al.*, 1974). Payne *et al.* (1974) did not follow seasonal trends but did find that values for these parameters were consistently higher June to September than January to March in the northern hemisphere.

The haematological parameters are moderately sensitive to climatic influences since the changes result in differing patterns of response and variations in level in successive years (Payne *et al.*, 1967).

The cause of the climatic effect is not clear; temperature is one factor that could be involved. Early work (Manresa *et al.*, 1934, 1939a, b) reported that the atmospheric temperature and haemoglobin levels were negatively correlated. The shape of the curve they reported was similar to the graphs obtained in the Massey units. Later workers (Rusoff *et al.*, 1954), plotted ambient temperature and haemoglobin levels over a period and obtained a graph which was consistent with a positive correlation between temperature and haemoglobin. Brody (1949) on the other hand recorded no differences associated with ambient temperature.

b) The influence of nutrition

Seasonal variation in nutrition is inherent in the all-grass grazing situation of the New Zealand Dairy industry where the quality and quantity of feed offered varies markedly according to the influence of climate with surges of grass growth occuring in the spring and autumn periods. The first report of a depression of haemoglobin levels due to diet was in monozygous twin calves fed a low protein diet (Greig and Boyne, 1956). Other workers have since confirmed this effect especially when it is associated with the onset of lactation (Hewett, 1974; Manston *et al.*, 1975; Treacher *et al.*, 1976). A shortage of protein in the diet of New Zealand cattle however, is not likely to be a limiting factor as the protein content of New Zealand pasture has been reported to be high enough to meet dietary needs (Johns, 1955; Hutton *et al.*, 1965). In this context it should be noted that high average plasma urea nitrogen levels were obtained with the cattle involved in this project (Table IV:1).

Whether energy intake is involved is not certain. Neither animal weights nor condition scores were kept during the course of the project but, on a subjective visual assessment of body condition, it would be the authors opinion that the cattle on Unit 1 were in the best overall state of nutrition yet had the lowest mean haemoglobin levels. Cattle on Unit 3 were subjected to the greatest nutritional stress as a result of other experimental procedures and the mean haemoglobin level on this unit was not different from that on Unit 2.

c) Lactation

A third potential cause of seasonal effects could result from the influence of lactation, particularly since calving in New Zealand is of a seasonal nature. On Units 2 and 3 and to a lesser extent Unit 1, the rise in the haematocrit and haemoglobin values that took place followed the spring calving which in all units had just

started by the time sampling began. It may be seen that the autumn calving group on Unit 1 were also calving during a period when the haematocrit and haemoglobin levels were rising.

To assess whether lactation had any effect on the levels of the haematocrit and haemoglobin the figures for the movement of these parameters in relation to weeks in milk should be studied (Figs. IV:5-8 and IV:23-26). Unit 3 was the first unit sampled; as a consequence few cows had calved at the first test and the effect of lactation on the sampled group would be least. On Units 1 and 2 however, where sampling commenced later, a larger proportion of the sampled group were lactating at this initial test yet the mean values obtained were very similar to those obtained for Unit 3. It seems reasonable to conclude from this that the fall in haematocrit and haemoglobin levels on Unit 3 at this time was predominantly a seasonal rather than a lactational effect (Figs. IV:3 and IV:21).

Although a fall in haemoglobin has been recorded as taking place at the start of lactation (Little , 1974; Hewett, 1974) in the U.K. and Sweden, this effect was not noted here. This is possibly the result of the very high protein content of pasture during the spring growth period in New Zealand with a fall occurring later when feed matures and readily digestible protein levels fall. The major changes in haemoglobin and haematocrit levels are therefore likely to be the result of seasonal changes in food quality and quantity rather than due to lactation.

Further evidence for the influence of lactation on the values obtained at the monthly sampling dates can be seen from a study of Figs. IV:9-11 and IV:27-29 for Unit 1 which has both an autumn and a spring calving group. At the start of the plots, which in both cases was in the spring when there was a considerable quantity of high protein food on offer, the haematocrit and haemoglobin values rise

although this rise was not as high in the spring calving group as in the autumn calving group. After a relatively small difference at the start of the plot where the autumn calving group is less than 1% higher in the haematocrit value, for example see Fig. IV:11, this difference in favour of the autumn calving group increases to over 2%, a change which could be interpreted as the effect of lactation in the spring calving group, i.e. as the spring surge of pasture occurs there is a rise in the haematocrit and haemoglobin values associated with the high level of available protein at this time (Payne *et al.*, 1973); this effect is however reduced in the spring calving group due to the depressing effect of lactation (Hewett, 1974; Little, 1974) and hence the curves between the two groups of animals diverge. Later the curves cross - an effect that is associated with the surge of autumn growth coinciding with peak lactation in the autumn calving group (and depressing the blood parameters) while the spring calving group are ceasing to lactate at this point in time.

The plot according to weeks in milk for the spring and autumn calving groups on Unit 1 produced rather different shaped curves (Figs. IV:12-14 and IV:30-32) and it looked initially that there was little or no relationship between the two groups. However the principal feature of each graph was a rise in haemoglobin and haematocrit. This was at week 28 in the autumn calving group (Fig. IV:12) and represented the period of spring growth. The rise in the spring calving group associated with the spring growth, which might be expected at the start of the plot, was almost non-existent and appears to have been modified by the demands of peak lactation. There is a rise at the end of the plot however, which would appear to be associated with the flush of growth occurring in the autumn (Fig. IV:13). The conclusion which may be drawn from this is that there is an effect due to lactation but the changes resulting from this are not as significant as those resulting from the seasonal effect. The latter is itself compounded

of a number of factors such as management, nutrition and climate all of which are associated under the all year round grazing system of seasonal farming as practised in New Zealand.

The influence of cow age on the haematological parameters differs between units and is difficult to interpret (Figs. IV:15-18 and IV:33-36). There appears to be little marked change due to age in both Units 1 and 3, a finding which is consistent with that reported by Schalm (1965) who stated that age had little influence on the haemoglobin level and haematocrit after the animals reached maturity. On Unit 2 there appeared to be an anaemia present in the two year old heifers, a result which could be associated with calving before maturity and the social pressure within the group restricting the feed intake of the younger smaller animals.

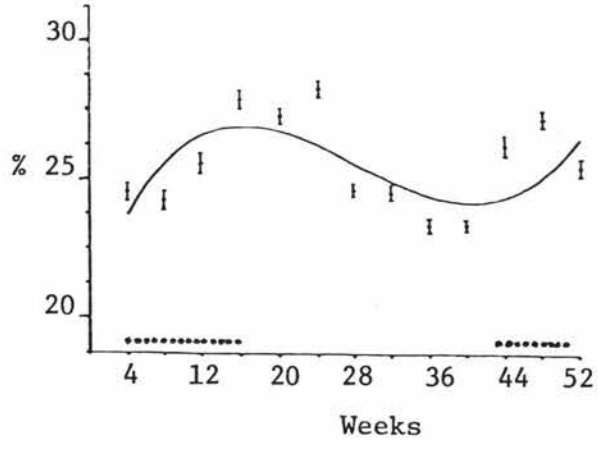


Figure IV:1

Massey No. 1 dairy unit
Haematocrit
Time in 4 week intervals

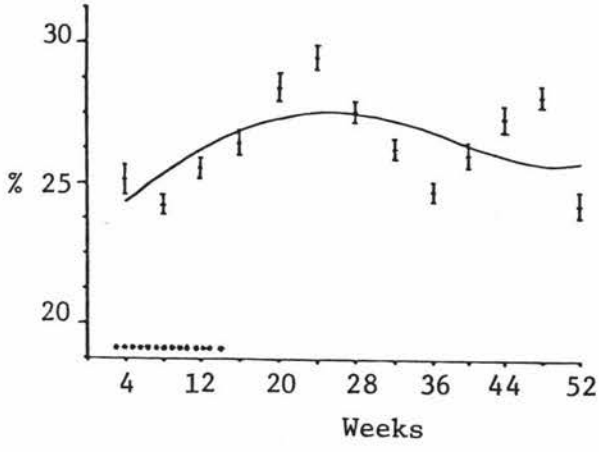


Figure IV:2

Massey No. 2 dairy unit
Haematocrit
Time in 4 week intervals

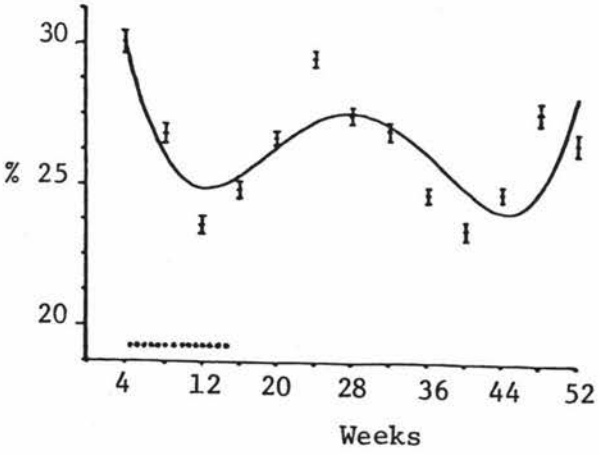


Figure IV:3

Massey No. 3 dairy unit
Haematocrit
Time in 4 week intervals

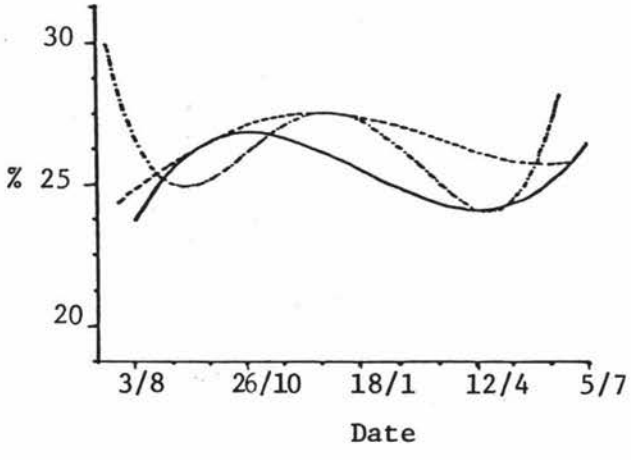


Figure IV:4

All 3 dairy units Massey
Haematocrit
Simultaneous plot

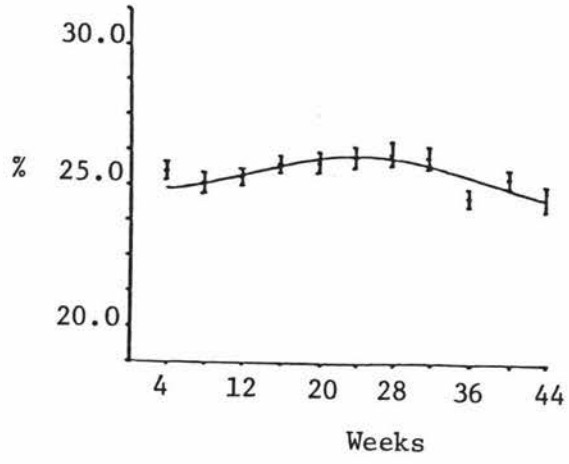


Figure IV:5

Massey No. 1 dairy unit
Haematocrit
Weeks in milk

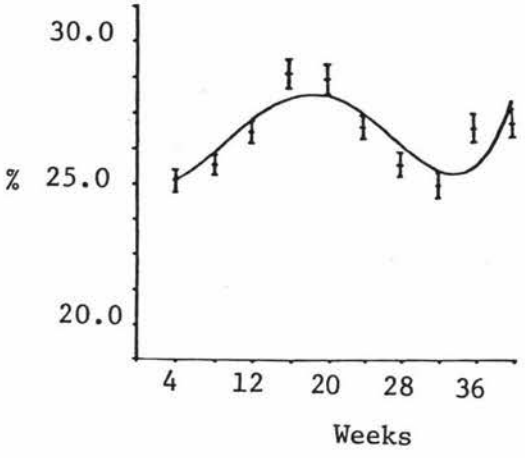


Figure IV:6

Massey No. 2 dairy unit
Haematocrit
Weeks in milk

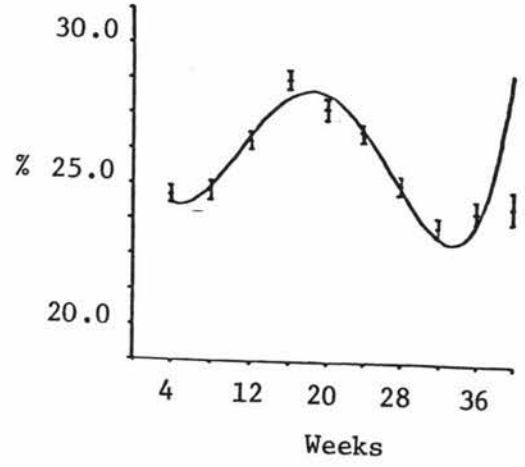


Figure IV:7

Massey No. 3 dairy unit
Haematocrit
Weeks in milk

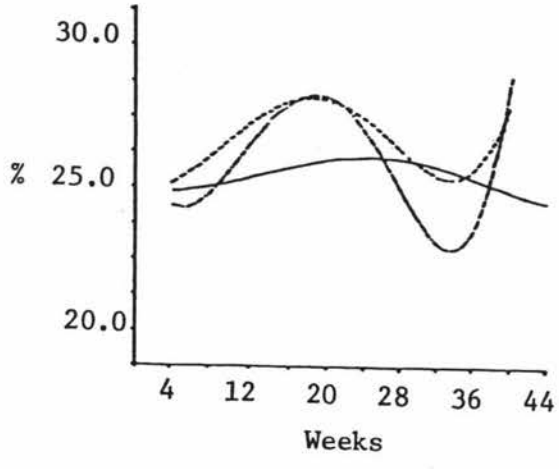


Figure IV:8

All 3 dairy units Massey
Haematocrit
Weeks in milk

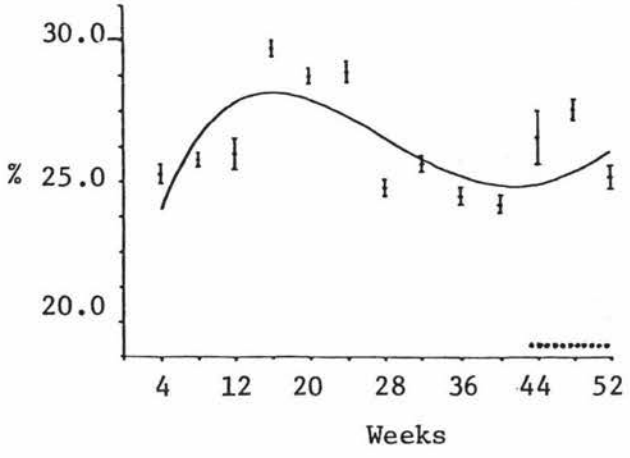


Figure IV:9

Massey No. 1 dairy unit
Autumn calving group
Haematocrit
Time in 4 week intervals

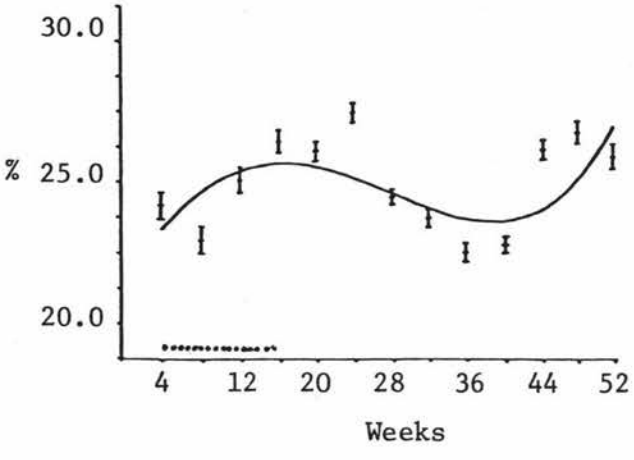


Figure IV:10

Massey No. 1 dairy unit
Spring calving group
Haematocrit
Time in 4 week intervals

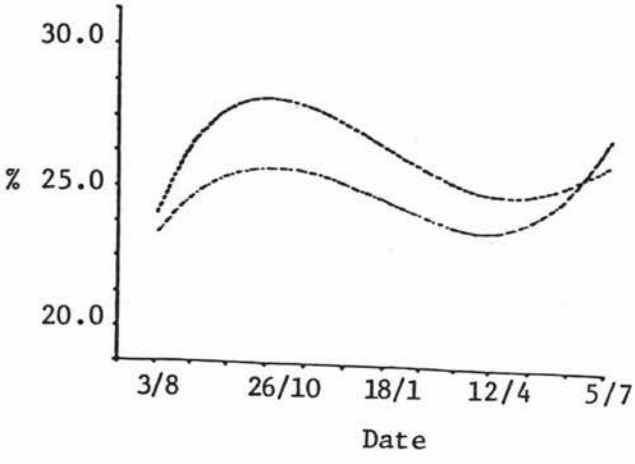


Figure IV:11

Massey No. 1 dairy unit
Autumn & spring calving group
Haematocrit
Time in 4 week intervals
Simultaneous plot

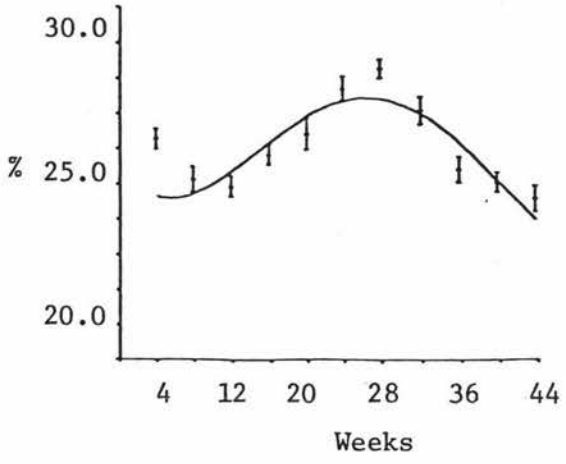


Figure IV:12

Massey No. 1 dairy unit
Autumn calving group
Haematocrit
Weeks in milk

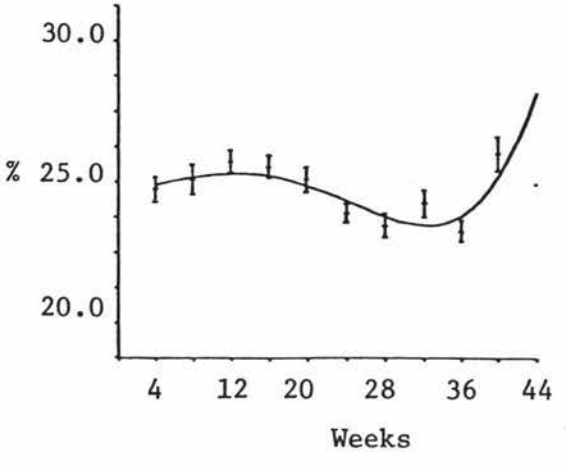


Figure IV:13

Massey No. 1 dairy unit
Spring calving group
Haematocrit
Weeks in milk

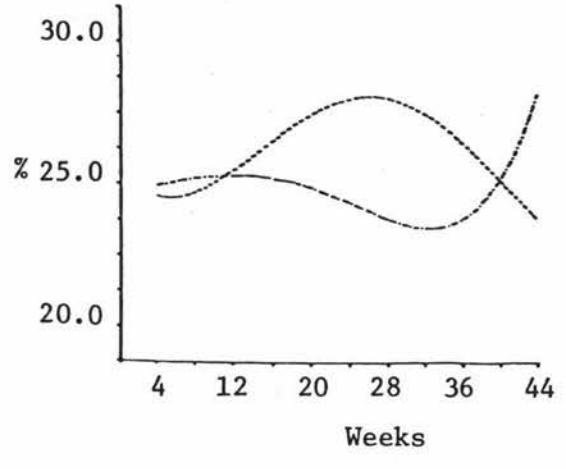


Figure IV:14

Massey No. 1 dairy unit
Autumn & spring calving group
Haematocrit
Weeks in milk
Simultaneous plot

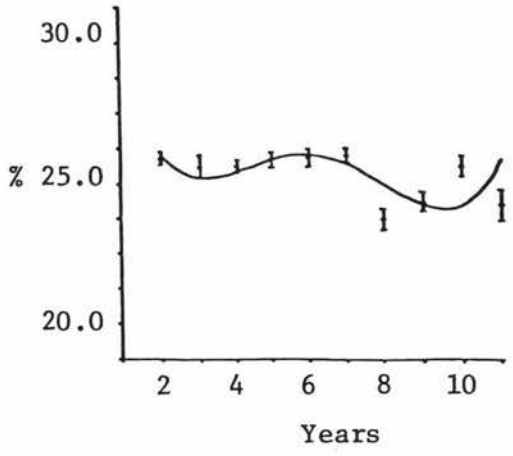


Figure IV:15

Massey No. 1 dairy unit
Haematocrit
Age

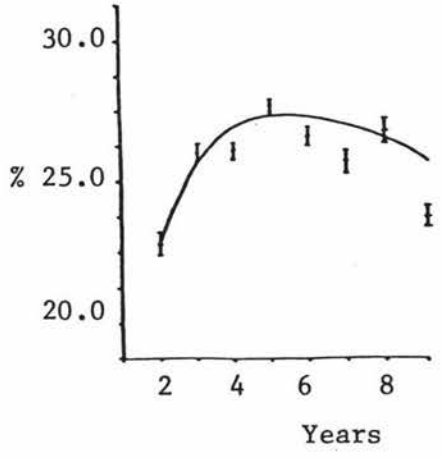


Figure IV:16

Massey No. 2 dairy unit
Haematocrit
Age

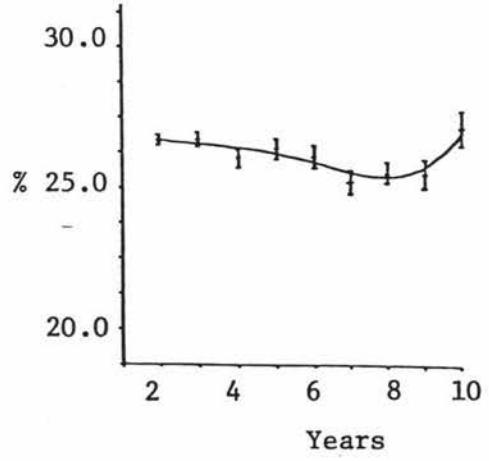


Figure IV:17

Massey No. 3 dairy unit
Haematocrit
Age

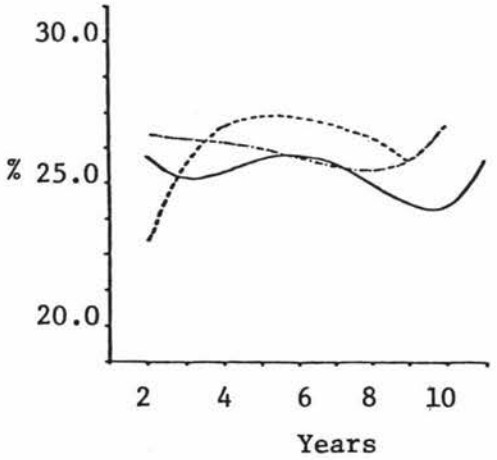


Figure IV:18

All 3 dairy units Massey
Haematocrit
Age

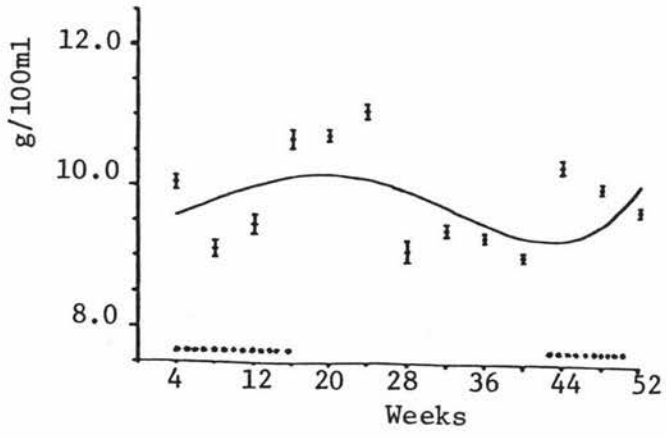


Figure IV:19

Massey No. 1 dairy unit
Haemoglobin
Time in 4 week intervals

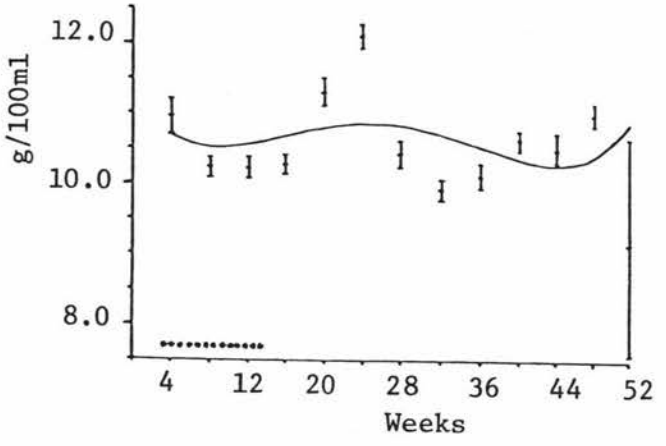


Figure IV:20

Massey No. 2 dairy unit
Haemoglobin
Time in 4 week intervals

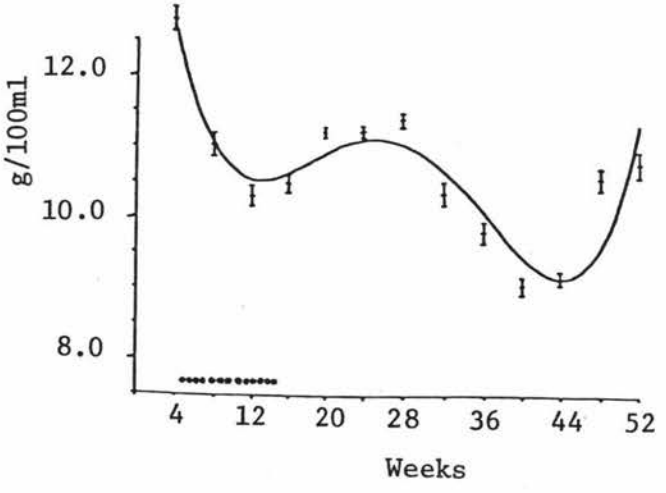


Figure IV:21

Massey No. 3 dairy unit
Haemoglobin
Time in 4 week intervals

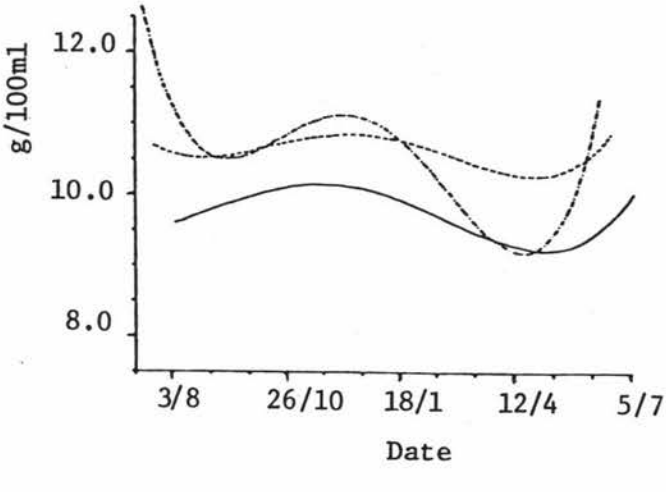


Figure IV:22

All 3 dairy units Massey
Haemoglobin
Simultaneous plot

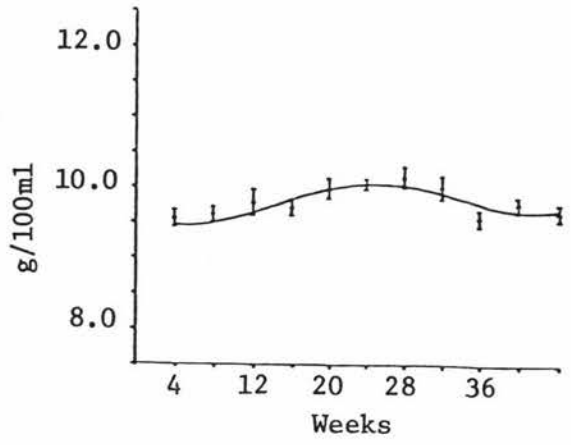


Figure IV:23

Massey No. 1 dairy unit
Haemoglobin
Weeks in milk

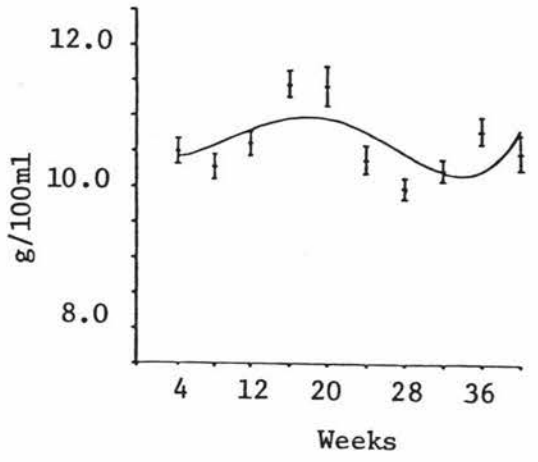


Figure IV:24

Massey No. 2 dairy unit
Haemoglobin
Weeks in milk

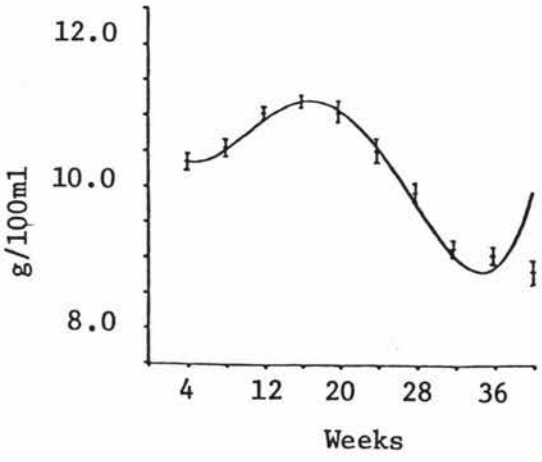


Figure IV:25

Massey No. 3 dairy unit
Haemoglobin
Weeks in milk

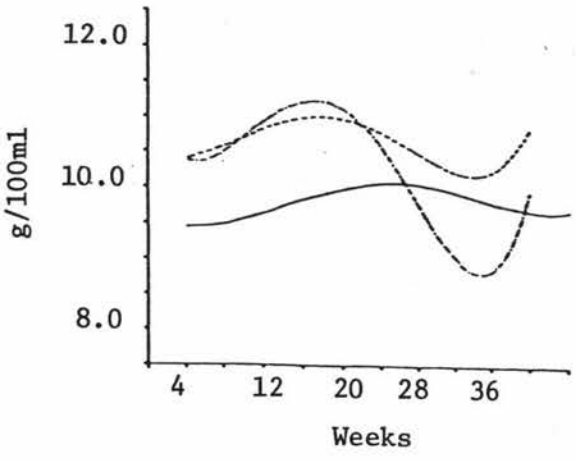


Figure IV:26

All 3 dairy units Massey
Haemoglobin
Weeks in milk

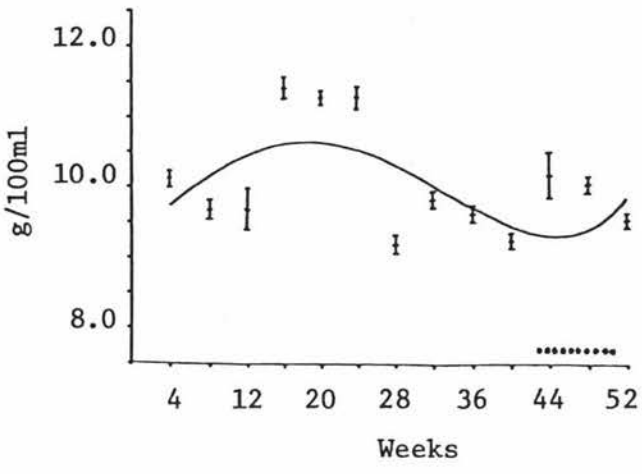


Figure IV:27

Massey No. 1 dairy unit
Autumn calving group
Haemoglobin
Time in 4 week intervals

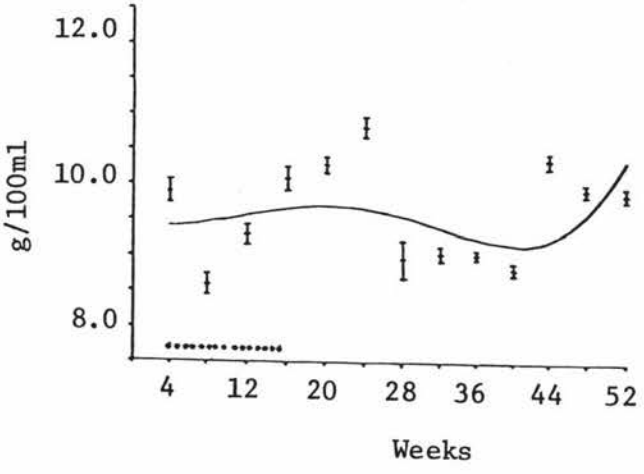


Figure IV:28

Massey No. 1 dairy unit
Spring calving group
Haemoglobin
Time in 4 week intervals

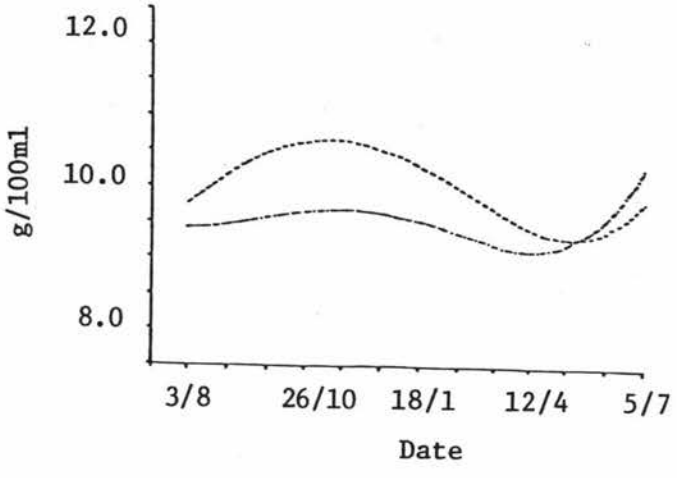


Figure IV:29

Massey No. 1 dairy unit
Autumn & spring calving groups
Haemoglobin
Time in 4 week intervals
Simultaneous plot

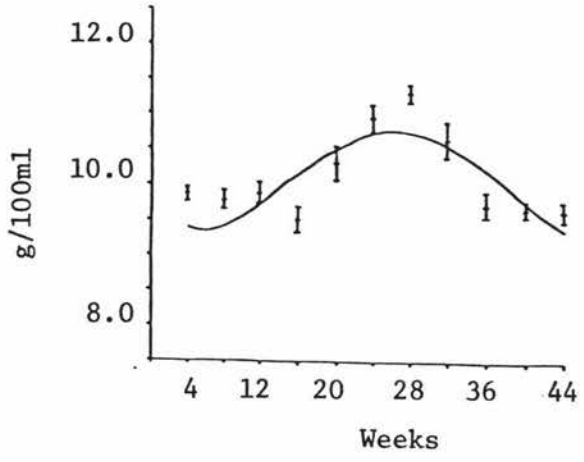


Figure IV:30

Massey No. 1 dairy unit
Autumn calving group
Haemoglobin
Weeks in milk

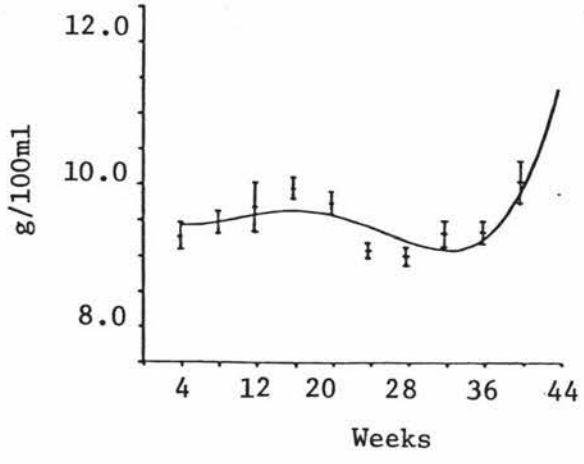


Figure IV:31

Massey No. 1 dairy unit
Spring calving group
Haemoglobin
Weeks in milk

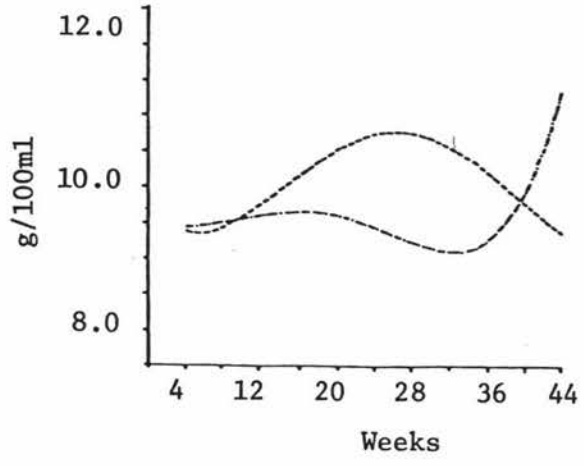


Figure IV:32

Massey No. 1 dairy unit
Autumn & spring calving groups
Haemoglobin
Weeks in milk
Simultaneous plot

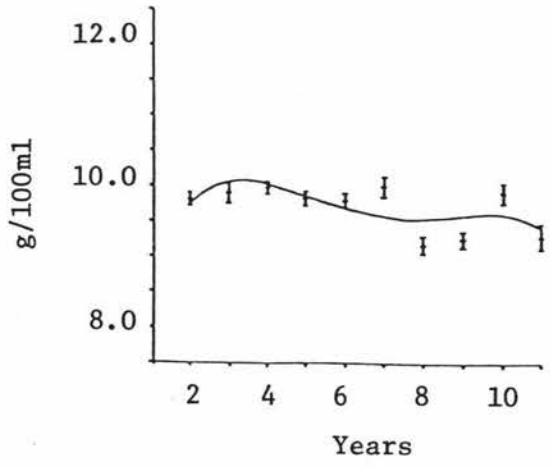


Figure IV:33

Massey No. 1 dairy unit
Haemoglobin
Age

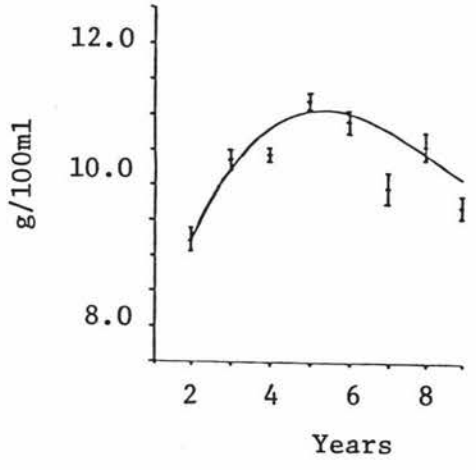


Figure IV:34

Massey No. 2 dairy unit
Haemoglobin
Age

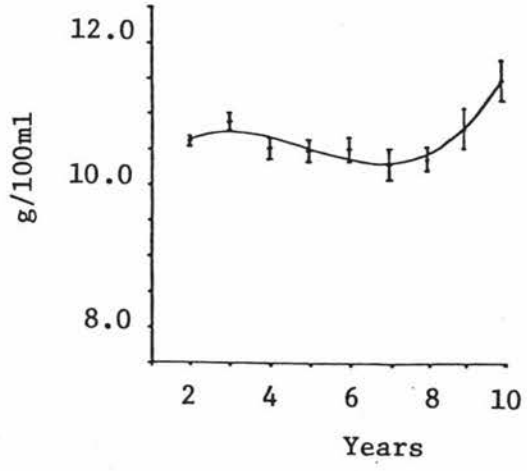


Figure IV:35

Massey No. 3 dairy unit
Haemoglobin
Age

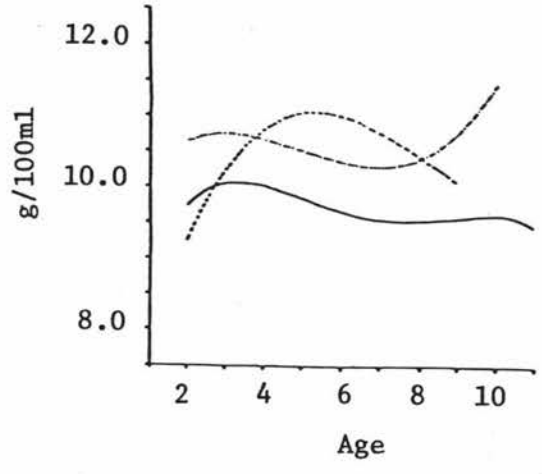


Figure IV:36

All 3 dairy units Massey
Haemoglobin
Age

Total Protein and Albumin

The mean total protein value for Unit 1 was much higher, that for Unit 3 very similar, and that for Unit 2 intermediate between Unit 1 and the U.K. figures for total protein levels (Table IV:1). There are two possible reasons which could explain this variation in mean serum total protein values: -

a) Nutrition: A number of workers have reported that a high or increasing digestible crude protein intake results in a high or increasing serum protein level, especially of the albumin fraction (see Review of Literature). Other components of the profile have also been claimed to be influenced by the level of protein nutrition.

   i) Urea nitrogen - While no direct measurements of protein intake were made during this project good evidence has been advanced that plasma urea nitrogen is a sensitive indicator of protein intake (see Review of Literature). The urea nitrogen values on Unit 1 were higher than those on Unit 2 which in turn were higher than those on Unit 3. This suggested that differences in serum total protein levels could have arisen by differences in the digestible crude protein intake on these different units.

   ii) Haemoglobin - Payne *et al.* (1974) also suggested that this parameter was an indicator of protein nutrition on a long term basis. In the investigation described in this thesis however, haemoglobin levels on Unit 1 were lower than on the other two units and much lower than the U.K. figures. This is contrary evidence to that suggested under (i) for differences in protein levels of nutrition between the Massey units.

   iii) Albumin - Serum albumin has been described as a more sensitive indicator of protein intake than serum

total protein (see Review of Literature). The level obtained on Unit 1 was very similar to that obtained in the U.K. but considerably lower than on Unit 3 (Table IV:1) yet Unit 3 during the course of the investigation was the unit that suffered most from feed deprivation. Again these findings would not support the contention that there were marked differences between the units in the level of protein nutrition that was offered.

One is forced to conclude therefore that the evidence from Part A of this project did not clearly establish that nutrition was an important cause of the differences between the results that were obtained.

b) Age: It is apparent from Table IV:1 that in the case of Unit 1 the increase in total protein over the U.K. figure must be due to an increased globulin fraction since the albumin levels were similar. One of the changes that has been reported in the literature (see Literature Review) is a decrease in the albumin fraction and an increase in the globulin fraction of the serum total protein with increasing age, most of the change occurring in the first five years of life and values fluctuating thereafter. The plot for total protein against age for Unit 1 (Fig . IV:51) showed this increase. It is not known whether there were age differences between the Massey Units and the animals from which the U.K. data were recorded; however there was a wider spread of age on Unit 1 than on Units 2 and 3. Examination of the plots for age (Fig. IV:54) however, indicated that serum total protein was higher on Unit 1 than on Units 2 and 3 at all ages suggesting that age alone did not provide the reason for the differences in total serum protein observed.

Continuing exposure to disease has been suggested as one of the causes of increasing globulin with increasing age (Rowlands, 1980a). Any increase in globulin on Unit 1 was unlikely to have been the consequence of repeated exposure to a wide variety of diseases as the author was

the attending veterinarian to the Unit and no continuing disease conditions were recorded at the time. Most other causes of change in serum total protein level result in a fall rather than a rise e.g. lactation, pregnancy and liver damage. Furthermore there was no evidence of total protein values being higher as a consequence of haemoconcentration since the haematocrit values were not elevated. The high total serum protein levels on Unit 1 therefore remain an enigma.

The plots for total protein (Figs. IV:37-40) and albumin (Figs. IV:55-58) against weeks from the start appeared to behave differently for each of the three units. On Unit 1 (Figs. IV:37 and 55) there was a similarity between the plot for serum total protein and the plot for albumin although the magnitude of the change was greater for total protein indicating that, by difference, most of the change was occurring in the globulin fraction. The shape of the curve was similar to the shape of the plot for protein content of pasture against time (Fig. IV:91) suggesting that the changes were a reflection of changes in the protein content of the diet.

Another possibility is that there was a change due to climatic effects. Temperature changes have been reported to cause alterations in the serum total protein level (Halliday *et al.*, 1969; McDowell *et al.*, 1969) and this may be the cause of alterations in the serum total protein that have been reported to occur with season (Payne *et al.*, 1972a; Rowlands *et al.*, 1974a). Whether this was one of the factors associated with alterations in the serum total protein that occurred on Unit 1 cannot be determined as alterations in the diet also occur in response to changes in the climate.

The plot for total protein against weeks in milk for the separate spring and autumn calving groups on Unit 1 (Figs. IV:48-50) followed a seasonal pattern and showed a rise in late summer corresponding to weeks 18-22 for the spring calving group (Fig. IV:49) and weeks 38-44 for the autumn calving group (Fig. IV:48). The changes in the plot of

albumin against weeks in milk (Figs. IV:66-68) did not show such a marked variation, confirming that most of the alterations in serum total protein level were in the globulin fraction.

The changes in the plots of serum total protein and albumin against weeks from the start for Unit 2 (Figs. IV:38 and 56) were quite different from the plots for Unit 1. The serum total protein rose to a plateau in summer and fell away from that level. The rise and fall appeared to be unrelated to changes in the protein content of the pasture (Fig. IV:91) on this unit. Since the albumin rise at approximately the peak of the curve for serum total protein accounts for a relatively small proportion of the change, the increases must have been produced mainly by changes in the globulin fraction.

The changes recorded in the plots for serum total protein and albumin against weeks from the start for Unit 3 (Figs. IV:39 and 57) were different from each other and from the plots for the other units with the changes in total protein levels being small when compared with those on the other units. There was some evidence of a dietary deficiency on this unit (Figs. IV:94 and 75) during the spring and early summer period when pasture protein levels would normally be high. This deficiency would decrease any potential response to increased pasture protein. The albumin plot against weeks from the start (Fig. IV:57) showed a rise to a high point at 20 weeks (summer) and then a steady fall for the remainder of the time. For the later part of the curve (week 32 onwards) the extent of the fall in albumin would have been enough to account for the fall seen in the serum total protein curve. By difference between the two there must have been some fall in globulin at weeks 4-12; this would have been due to the demands of lactation when dietary intake was not adequate.

Only minor changes could be seen in the total protein plots against weeks in milk for Units 1 and 2 (Figs. IV:41-42). There was a change in the graph shape for Unit 3 in its terminal stages (weeks 30-40) (Fig. IV:43). The feed

supplement given to the stock over that period could account for this.

The plot for albumin against weeks in milk (Figs. IV:59-62) was very similar to that for total protein on Unit 1. However quite pronounced changes occurred on Units 2 and 3 where there was a fall in the latter part of lactation for Unit 2 and for the greater part of lactation on Unit 3. The inadequate, and with the supplementary feed, possibly unbalanced diet on Unit 3 may have accounted for the changes on this unit as during the later stages of lactation milk production has little influence on albumin levels according to Hewett (1974). The changes observed on Unit 2 cannot be accounted for.

The effect of lactation on serum total protein was confirmed by studying the plots against time in four week intervals for the separate spring and autumn calving groups on Unit 1 (Fig. IV:45-47). The total protein in the spring calving group was lower only at the later stages of pregnancy and early lactation, tending to confirm that lactation had little effect on serum total protein levels and that the fall in globulin was the result of immunoglobulins entering the udder during the secretion of colostrum.

The plots for albumin against weeks from the start for the separate spring and autumn calving groups (Figs. IV:63-65) showed the spring calving group to be lower at the start of the spring though not markedly so. This could have been because the high protein content of the spring pasture was able to sustain serum albumin levels in the face of the demands of lactation. The autumn calving group showed a greater fall after calving as the pasture protein was not as high as in the spring (Fig. IV:91). The serum albumin fell as a result of this and then recovered as the peak of lactation passed.

Total protein appeared to increase with age (Figs. IV:51-54) although plots for the different units showed some irregularity - this could have been a function of the relatively small samples in each age class involved. The rise probably resulted from an increase in globulin (principally gammaglobulin) with age up to about 5 years (see Review of Literature). This is frequently accompanied by a fall in albumin. However the plots for albumin levels against age (Figs. IV:69-72) showed no consistent pattern in this part of the project.

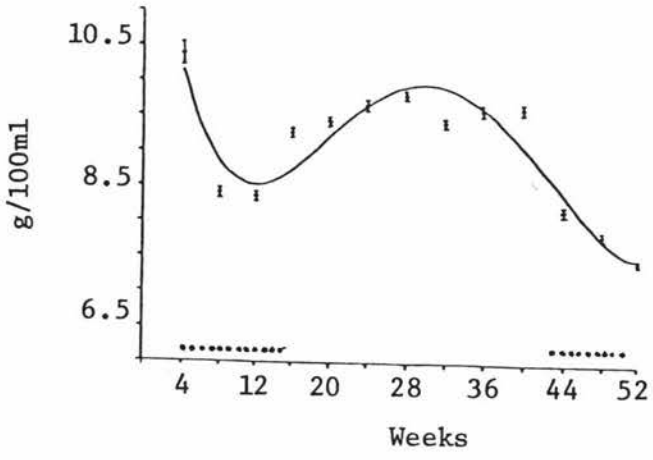


Figure IV:37

Massey No. 1 dairy unit
Total Protein
Time in 4 week intervals

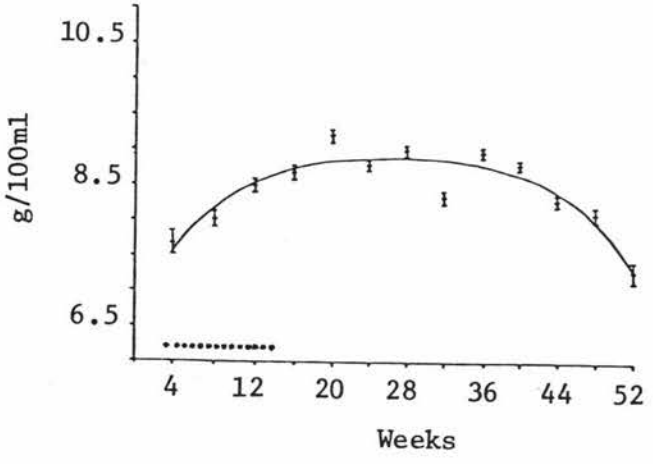


Figure IV:38

Massey No. 2 dairy unit
Total Protein
Time in 4 week intervals

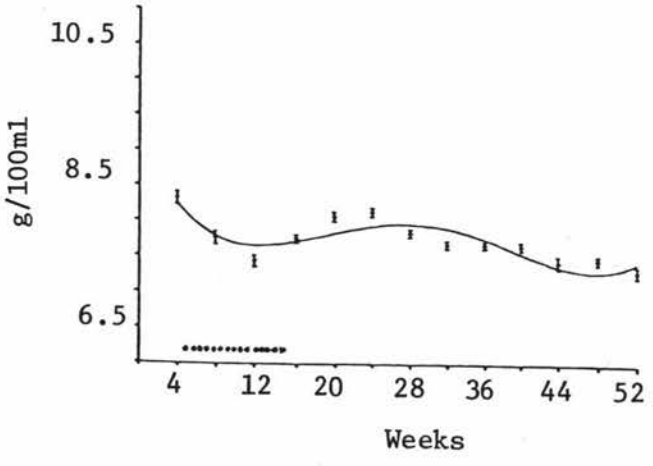


Figure IV:39

Massey No. 3 dairy unit
Total Protein
Time in 4 week intervals

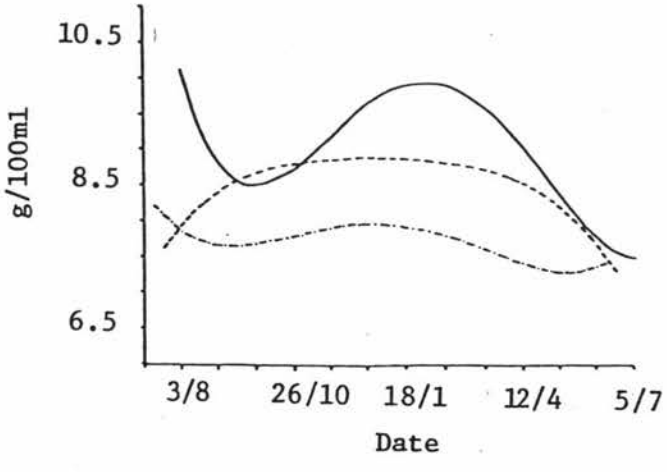


Figure IV:40

All 3 dairy units Massey
Total Protein
Simultaneous plot

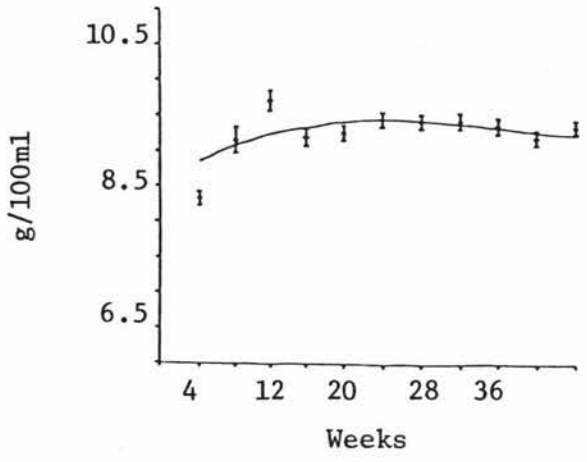


Figure IV:41

Massey No. 1 dairy unit
Total Protein
Weeks in milk

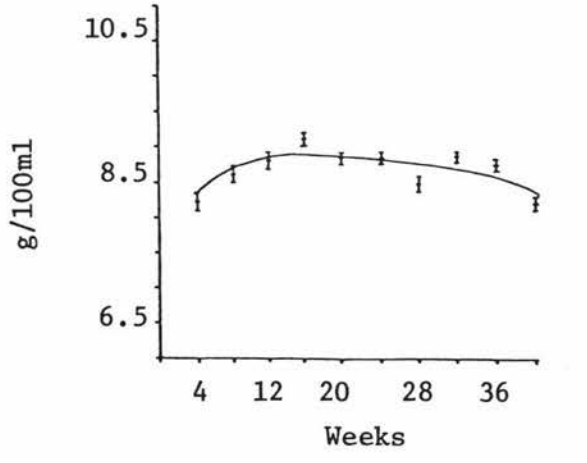


Figure IV:42

Massey No. 2 dairy unit
Total Protein
Weeks in milk

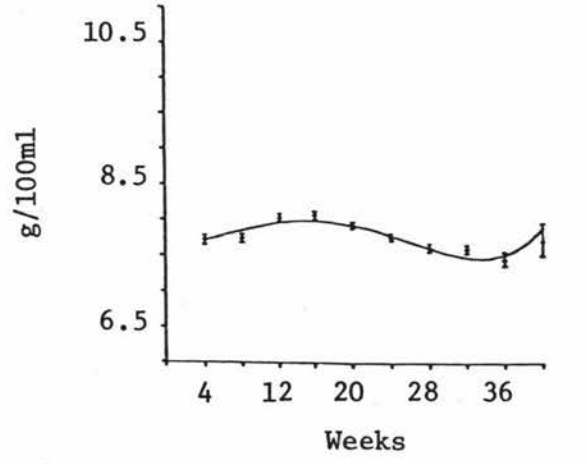


Figure IV:43

Massey No. 3 dairy unit
Total Protein
Weeks in milk

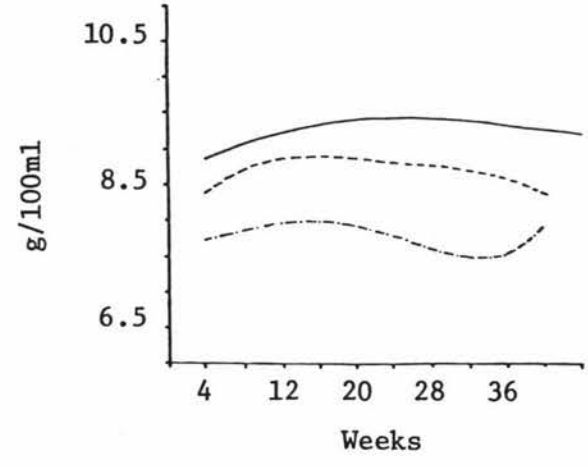


Figure IV:44

All 3 dairy units Massey
Total Protein
Weeks in milk

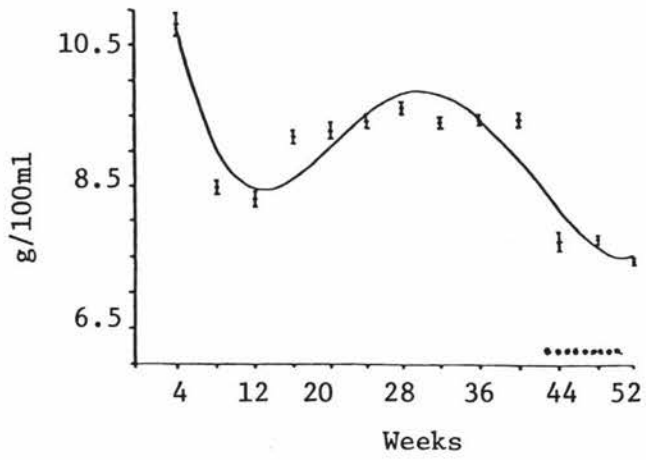


Figure IV:45

Massey No. 1 dairy unit
Autumn calving group
Total protein
Time in 4 week intervals

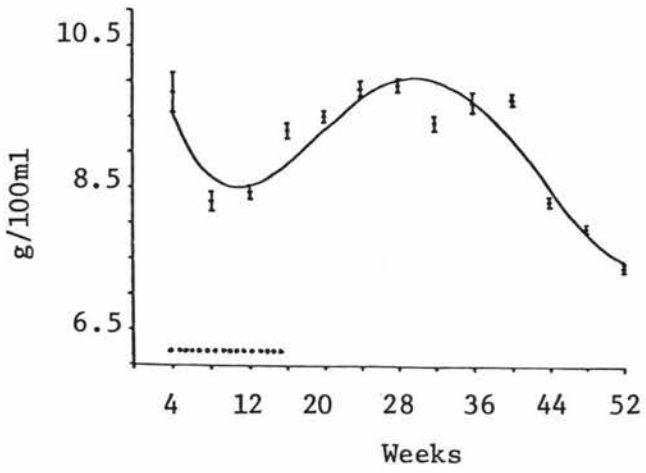


Figure IV:46

Massey No. 1 dairy unit
Spring calving group
Total protein
Time in 4 week intervals

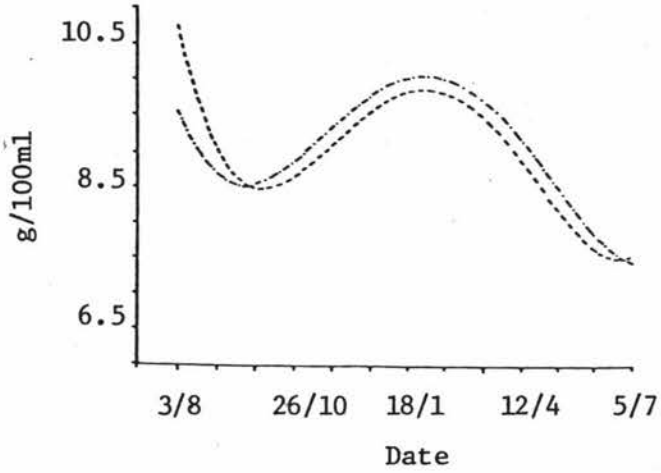


Figure IV:47

Massey No. 1 dairy unit
Autumn & spring calving groups
Total protein
Time in 4 week intervals
Simultaneous plot

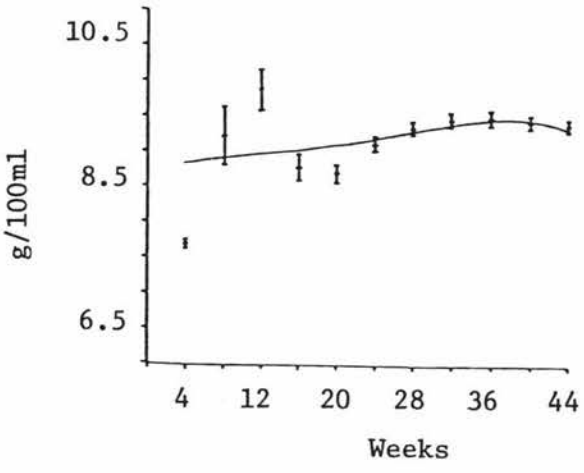


Figure IV:48

Massey No. 1 dairy unit
Autumn calving group
Total protein
Weeks in milk

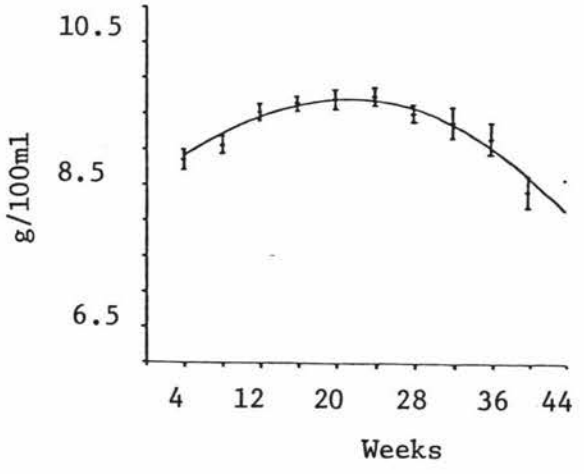


Figure IV:49

Massey No. 1 dairy unit
Spring calving group
Total protein
Weeks in milk

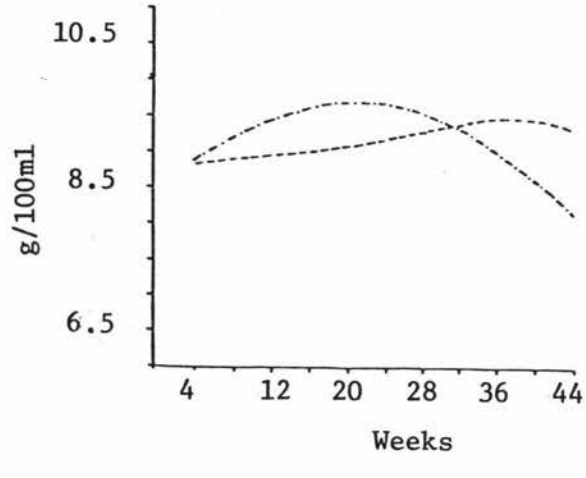


Figure IV:50

Massey No. 1 dairy unit
Autumn & spring calving groups
Total protein
Weeks in milk
Simultaneous plot

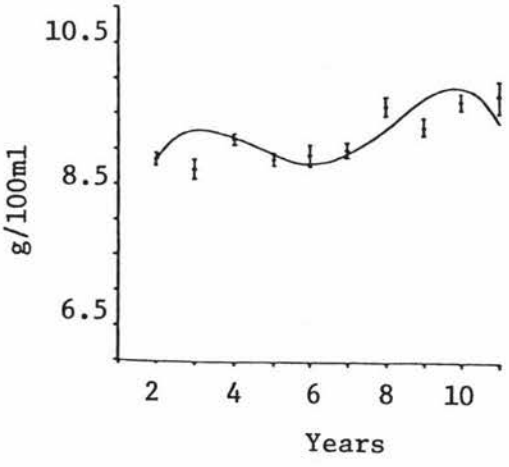


Figure IV:51

Massey No. 1 dairy unit
Total protein
Age

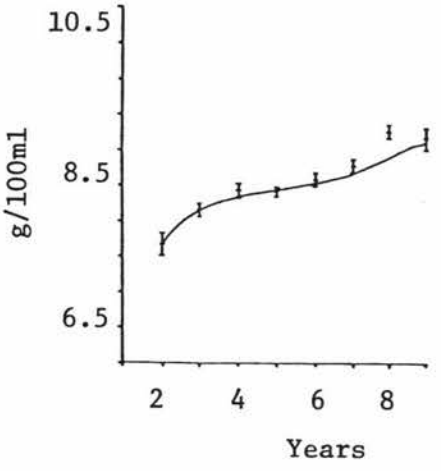


Figure IV:52

Massey No. 2 dairy unit
Total protein
Age

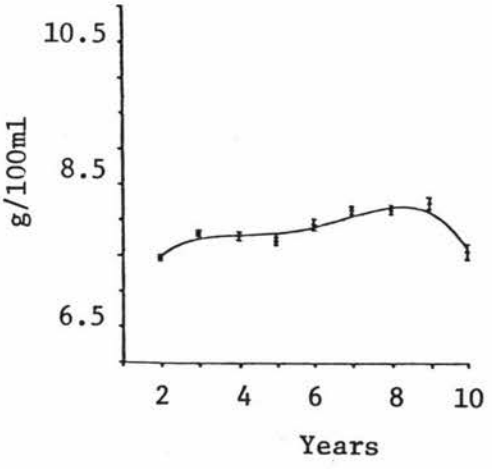


Figure IV:53

Massey No. 3 dairy unit
Total protein
Age

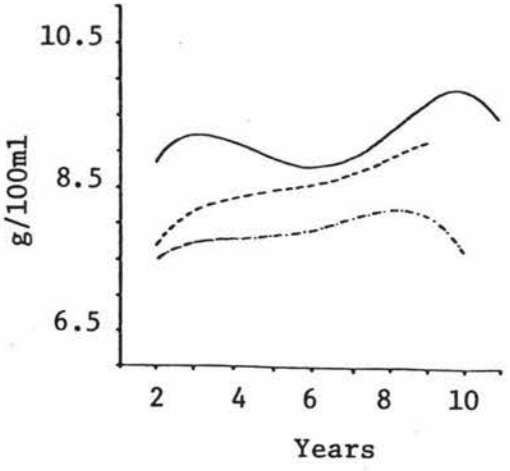


Figure IV:54

All 3 dairy units Massey
Total protein
Age

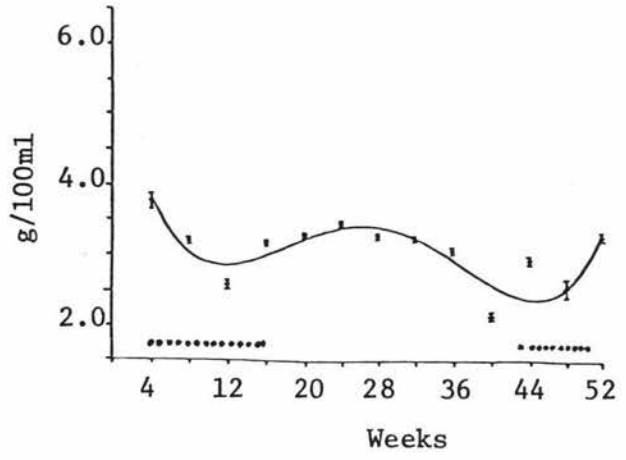


Figure IV:55

Massey No. 1 dairy unit
Albumin
Time in 4 week intervals

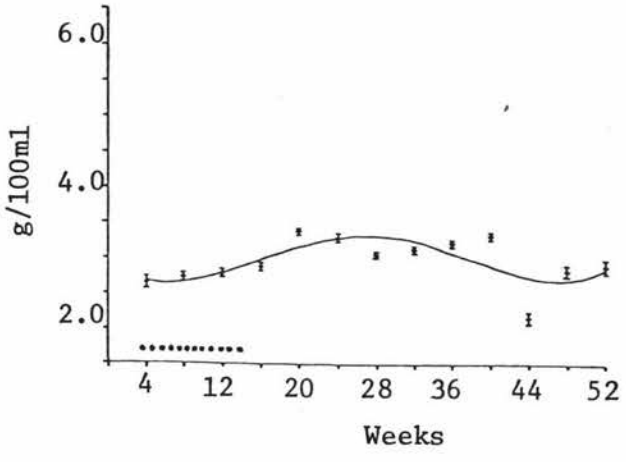


Figure IV:56

Massey No. 2 dairy unit
Albumin
Time in 4 week intervals

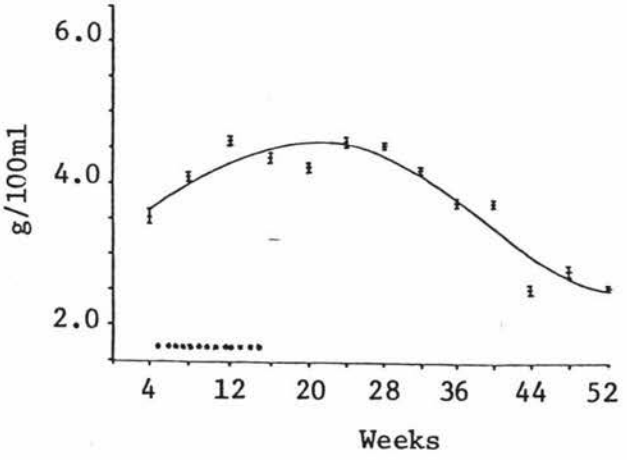


Figure IV:57

Massey No. 3 dairy unit
Albumin
Time in 4 week intervals

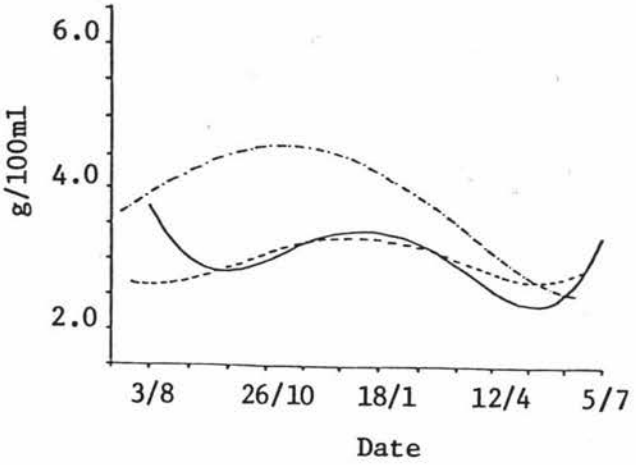


Figure IV:58

All 3 dairy units Massey
Albumin
Simultaneous plot

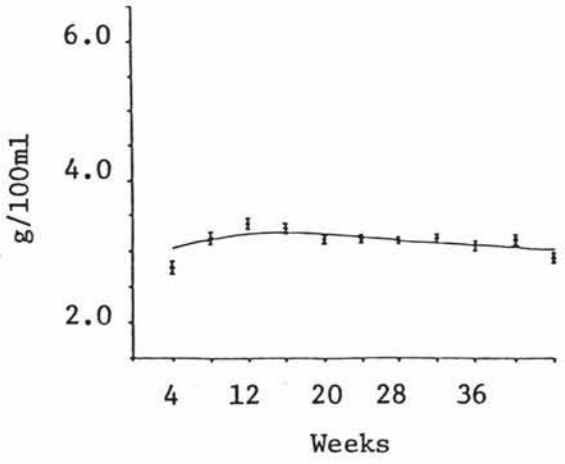


Figure IV:59

Massey No. 1 dairy unit
Albumin
Weeks in milk

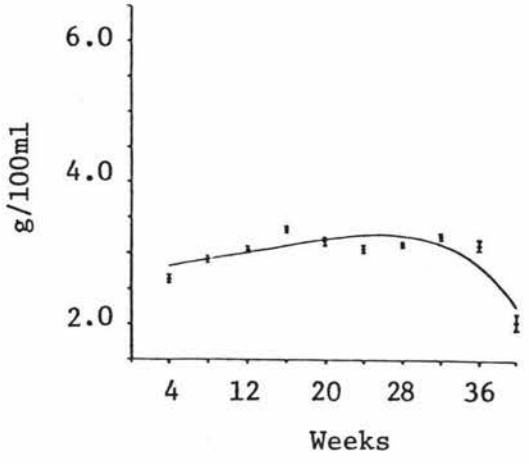


Figure IV:60

Massey No. 2 dairy unit
Albumin
Weeks in milk

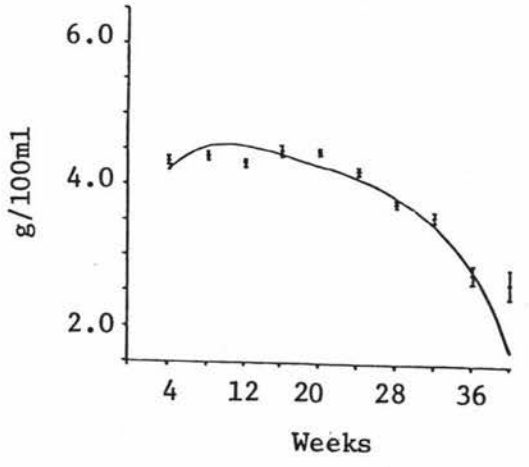


Figure IV:61

Massey No. 3 dairy unit
Albumin
Weeks in milk

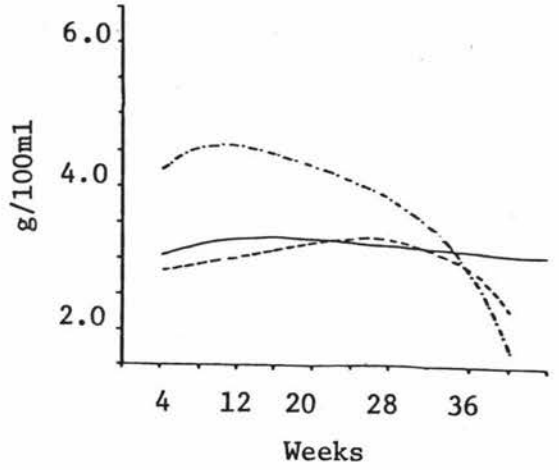


Figure IV:62

All 3 dairy units Massey
Albumin
Weeks in milk

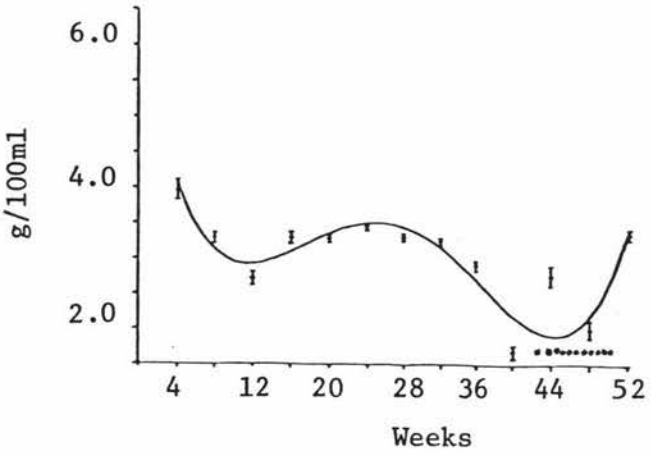


Figure IV:63

Massey No. 1 dairy unit
Autumn calving group
Albumin
Time in 4 week intervals

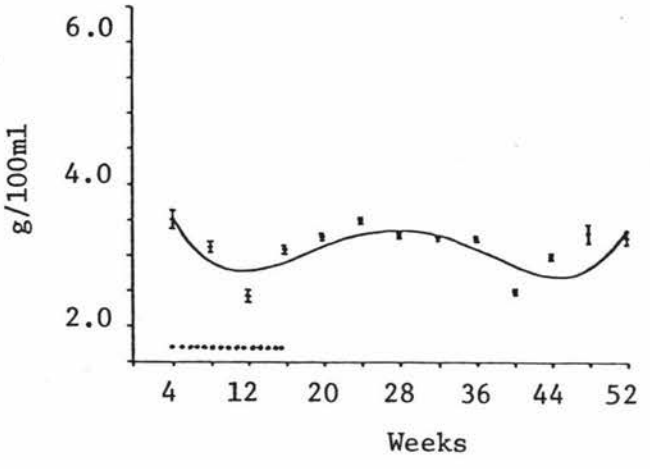


Figure IV:64

Massey No. 1 dairy unit
Spring calving group
Albumin
Time in 4 week intervals

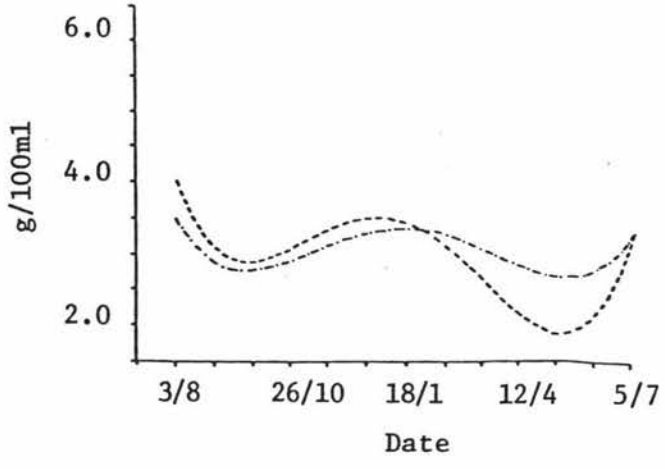


Figure IV:65

Massey No. 1 dairy unit
Autumn & spring calving groups
Albumin
Time in 4 week intervals
Simultaneous plot

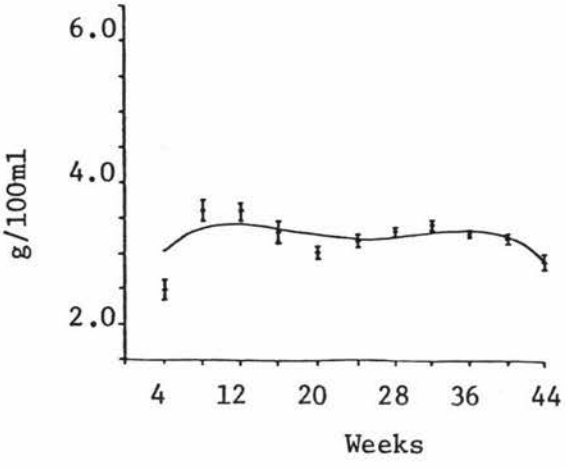


Figure IV:66

Massey No. 1 dairy unit
Autumn calving group
Albumin
Weeks in milk

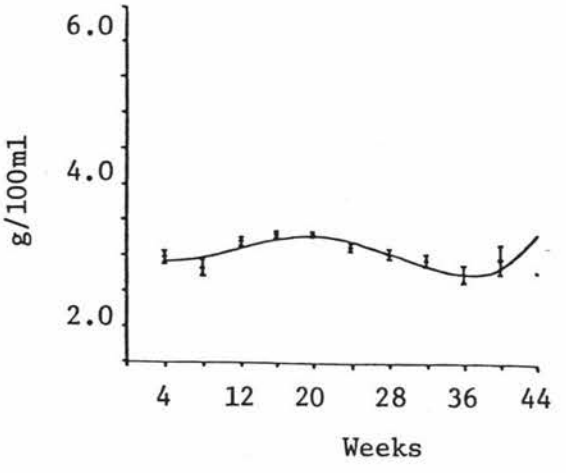


Figure IV:67

Massey No. 1 dairy unit
Spring calving group
Albumin
Weeks in milk

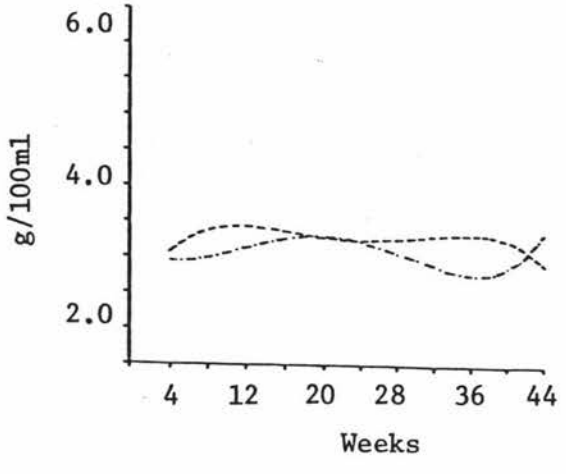


Figure IV:68

Massey No. 1 dairy unit
Autumn & spring calving groups
Albumin
Weeks in milk
Simultaneous plot

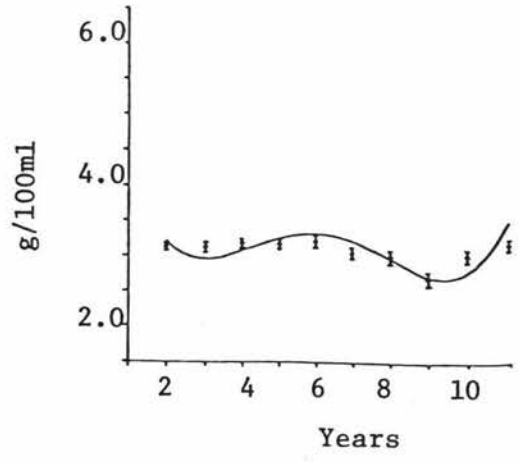


Figure IV:69

Massey No. 1 dairy unit
Albumin
Age

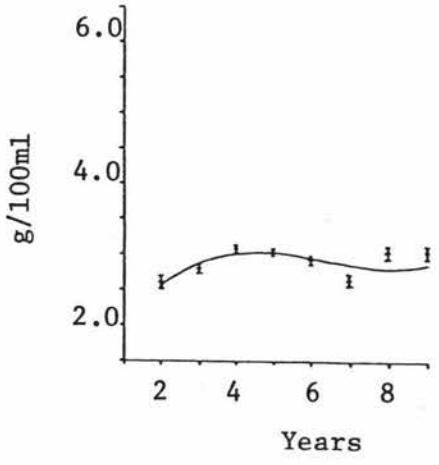


Figure IV:70

Massey No. 2 dairy unit
Albumin
Age

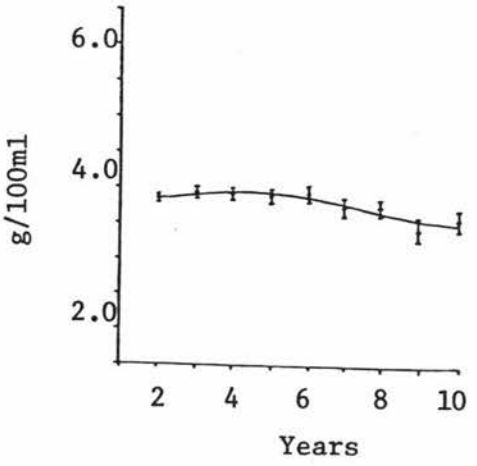


Figure IV:71

Massey No. 3 dairy unit
Albumin
Age

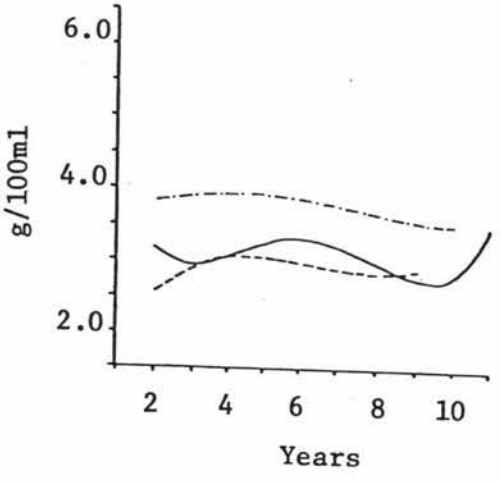


Figure IV:72

All 3 dairy units Massey
Albumin
Age

Urea Nitrogen

The results obtained for urea nitrogen for the three Massey Dairy Units were all higher than the U.K. figures (Table IV:1). This difference may have arisen because of the method of calculation. The test for urea in this project was performed on plasma, but it is not clear if the U.K. project used blood or plasma. Since their glucose measurements were on blood, and it is likely that a simultaneous assay was used for both glucose and urea, it seems reasonable to assume that their urea values were blood levels. If this is the case a correction factor would need to be applied to permit direct comparison between the U.K. figure and those obtained in this project. Although no correction factor has been published Baron (1969) states that plasma glucose is almost 14% higher than blood glucose and plasma urea is about 8% higher than blood urea. This would mean that if the U.K. urea assay was performed on whole blood, the corrected level for plasma would be 16-17mg/100ml, lower than the level obtained on Units 1 and 2 at Massey.

An alternative explanation for the Massey results being higher than the U.K. results could be associated with the diet that the cattle were receiving since it has been reported on several occasions (see Review of Literature) that plasma urea nitrogen changes fairly rapidly with the level of protein in the diet. Sixteen percent of protein in the diet is generally considered to be adequate to meet all demands of cattle (McCay, 1931); this level is usually available on mixed New Zealand pasture even when the protein content is at its lowest. Johns (1955) gives figures from which a chart of the protein content of the sward can be drawn (Fig. IV:91); at the point when pasture content of protein would be expected to be low (28-36 weeks after the start in Fig. IV:74) the serum values recorded for urea nitrogen were 15.5mg/100ml, a figure which is similar to the U.K. mean (Table IV:1). At other times the protein levels are high, especially when there are flushes of growth (Johns, 1955; Hutton *et al.*, 1965).

This is demonstrated in the plots for Units 1 and 2 (Figs. IV:73 & 74) both of which show peaks for urea nitrogen that are similar to the pasture protein peaks (Figs. IV:91). The absence of a small mid January rise in urea nitrogen values on these units, which would correspond with the January peak in Fig. IV:91 probably reflects the lack of soil moisture and suitable growing conditions during the summer over which the Massey data was collected.

The dissimilar plot of urea nitrogen for Unit 3 (Fig. IV:75) requires explanation. Initially, urea nitrogen values were all low, even during the period of spring growth. (This is unlikely to be explained as experimental error because of the assay method used - see Materials & Methods pp 134 & 140). An elevation of plasma urea nitrogen levels similar to those found on other units did not occur until after the initiation of supplementary feeding. Reference to the plot for glucose for this unit (Fig. IV:94) shows that the glucose level was falling very steadily during the time of lower plasma urea nitrogen levels. Thus the likely explanation for both results is that the levels of both protein and energy in the diet at this time were low. Any free ammonia produced in the rumen by plant protein breakdown would be utilised very rapidly by the rumen microflora as protein for their own energy sources. As a consequence, rumen ammonia and the resulting plasma urea nitrogen levels would be decreased.

A further possibility which could have resulted in the depression of the U.K. figure for urea nitrogen could be associated with carbohydrate supplementation (Payne *et al.*, 1973, 1974). It has been reported that this results in lower urea nitrogen levels in cattle on diets of equal protein content (Tillman & Sidhu, 1969).

The standard deviations of the means for urea nitrogen were also much greater for the Massey Dairy Units than was found in the U.K. (Table IV:1). This is most likely the result of less control over both quality and quantity of diet consumed in the New Zealand free grazing situation.

Data for urea nitrogen in Table IV:1 illustrates some of the shortcomings of the statistical methods used to analyse this information. In particular for Unit 3 it can be seen that 2 standard deviations below the mean approaches a value of zero. This is the result of a population distribution with a large number of values slightly lower than the mean being balanced by a few with much higher values. Such a skew distribution affects the ability of the result to predict abnormal values under the statistical method used in this project since it is a method designed for normal population distributions.

The plots for urea nitrogen levels against weeks in milk (Figs. IV:77-80) follow a similar trend to those for time of year except that the variation in urea nitrogen levels on Unit 1 is very low. This could be explained by the relatively spread calving on this unit so that, for example, some of the herd in their sixteenth week of lactation will have access to pasture high in protein and some to pasture low in protein with a consequent levelling out in the values of serum urea nitrogen obtained. The plots for urea nitrogen against weeks in milk for the separate spring and autumn calving groups on Unit 1 (Figs. IV:84-86) illustrate this effect. In Units 2 and 3 on the other hand where calving is restricted to the late winter and spring period, changes in urea nitrogen level with weeks in milk tend to mirror those for season. It is not known why there was a relatively consistent difference in the mean serum levels of urea nitrogen for the autumn and spring calving herds on Unit 1 irrespective of season (see the parallel nature of the curves in Figs. IV:81-83). Some characteristic of the animals within each of the two samples, possibly an inherited characteristic (see Part C p 273) may provide the explanation.

No significant age effect on urea nitrogen level was noted (Figs. IV: 87-90).

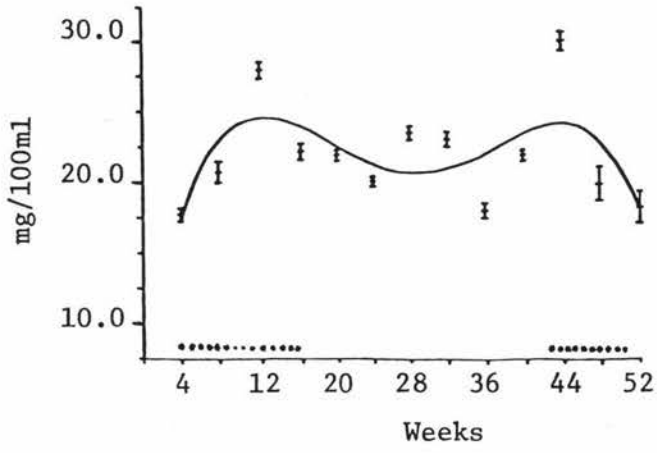


Figure IV:73

Massey No. 1 dairy unit
Urea nitrogen
Time in 4 week intervals

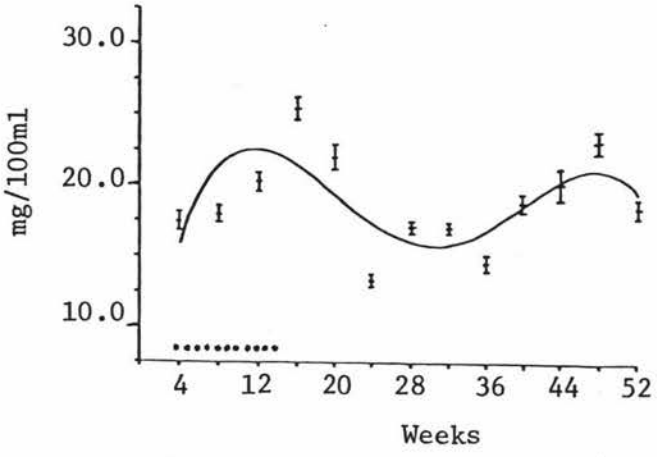


Figure IV:74

Massey No. 2 dairy unit
Urea nitrogen
Time in 4 week intervals

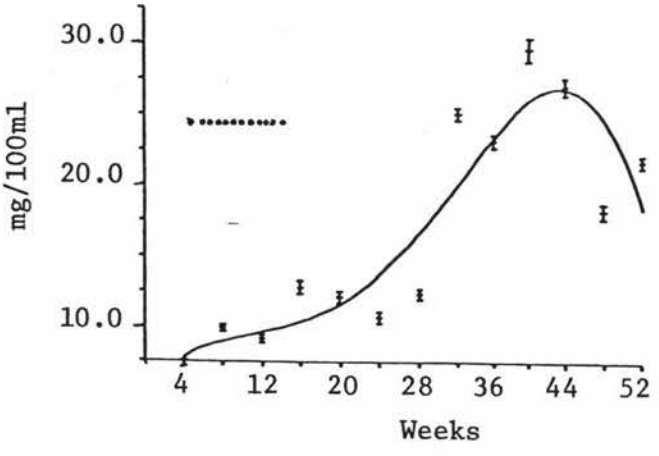


Figure IV:75

Massey No. 3 dairy unit
Urea nitrogen
Time in 4 week intervals

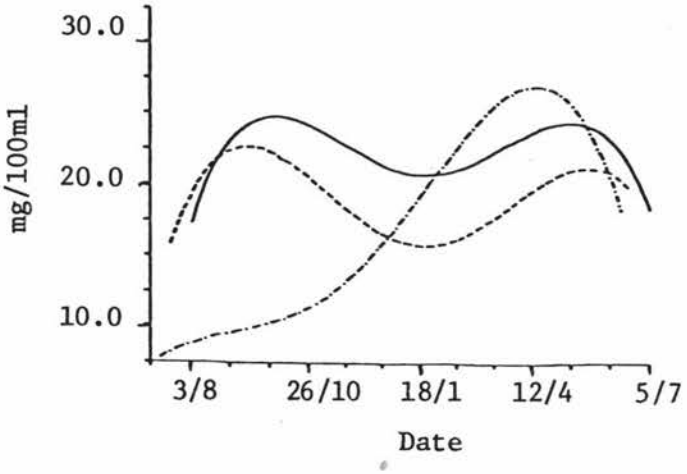


Figure IV:76

All 3 dairy units Massey
Urea nitrogen
Simultaneous plot

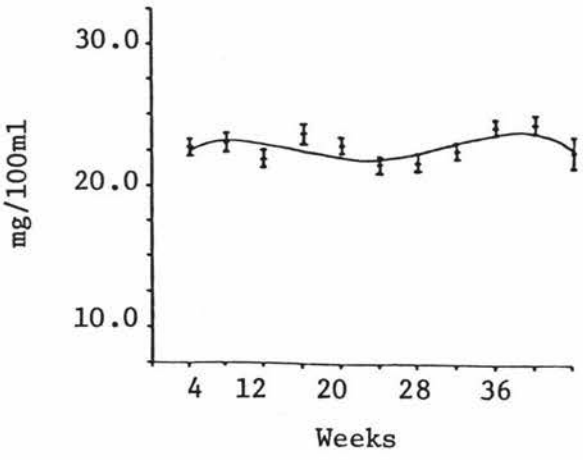


Figure IV:77

Massey No. 1 dairy unit
Urea Nitrogen
Weeks in milk

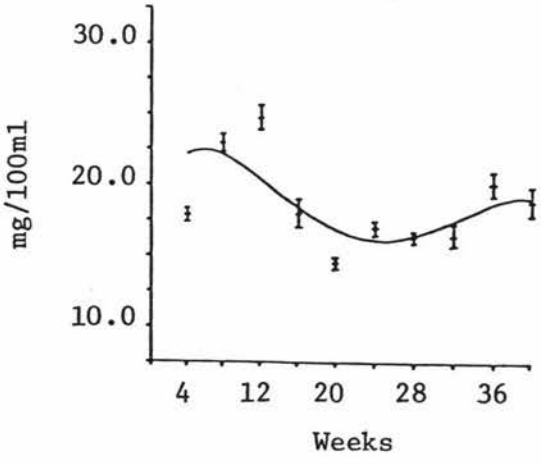


Figure IV:78

Massey No. 2 dairy unit
Urea Nitrogen
Weeks in milk

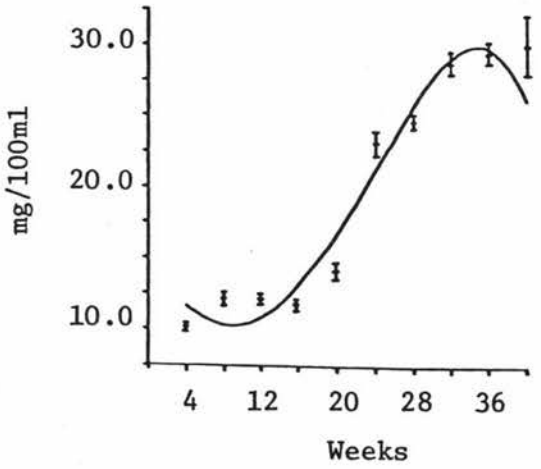


Figure IV:79

Massey No. 3 dairy unit
Urea Nitrogen
Weeks in milk

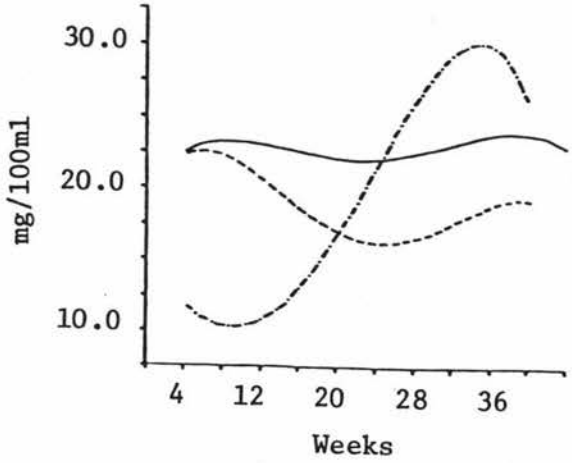


Figure IV:80

All 3 dairy units Massey
Urea Nitrogen
Weeks in milk

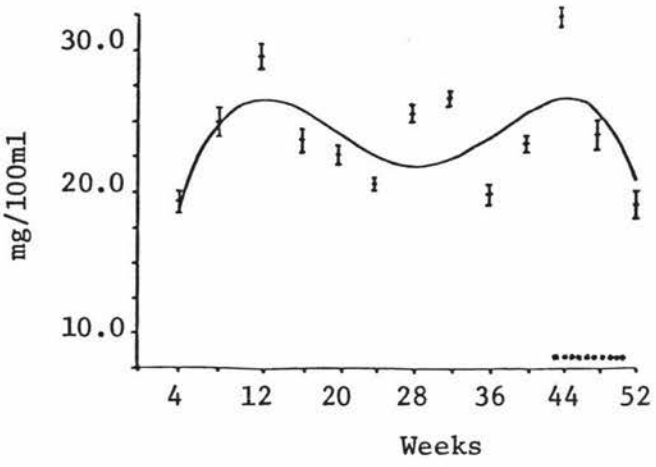


Figure IV:81

Massey No. 1 dairy unit
Autumn calving group
Urea nitrogen
Time in 4 week intervals

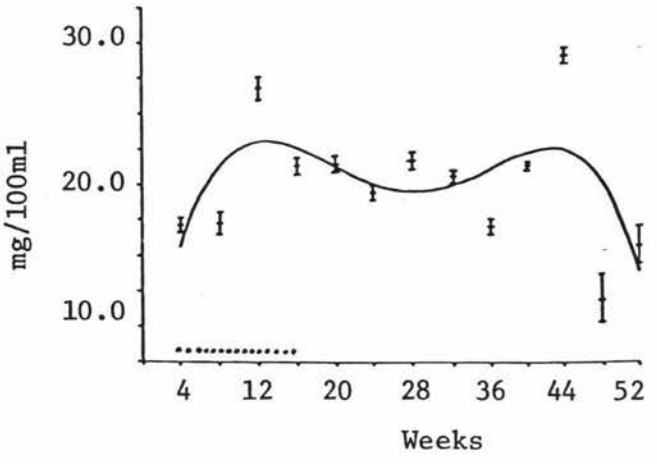


Figure IV:82

Massey No. 1 dairy unit
Spring calving group
Urea nitrogen
Time in 4 week intervals

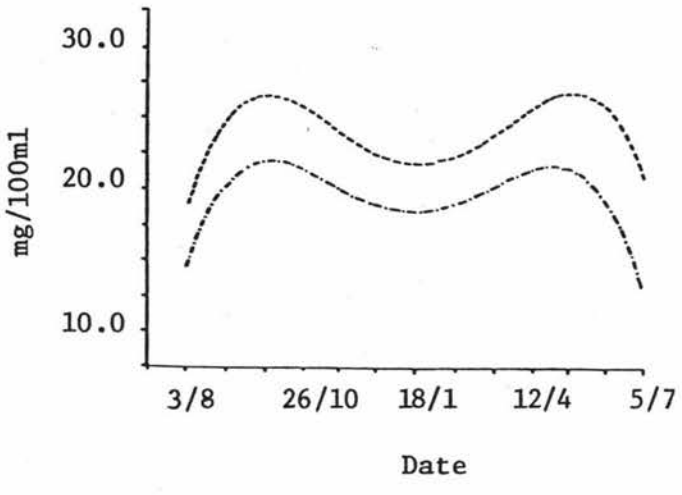


Figure IV:83

Massey No. 1 dairy unit
Autumn & spring calving groups
Urea nitrogen
Time in 4 week intervals
Simultaneous plot

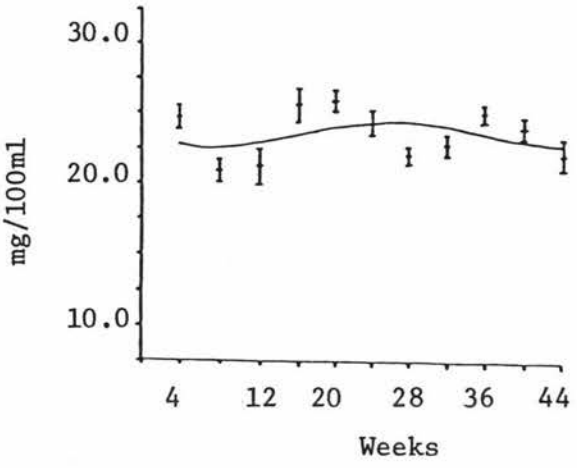


Figure IV:84

Massey No. 1 dairy unit
Autumn calving group
Urea nitrogen
Weeks in milk

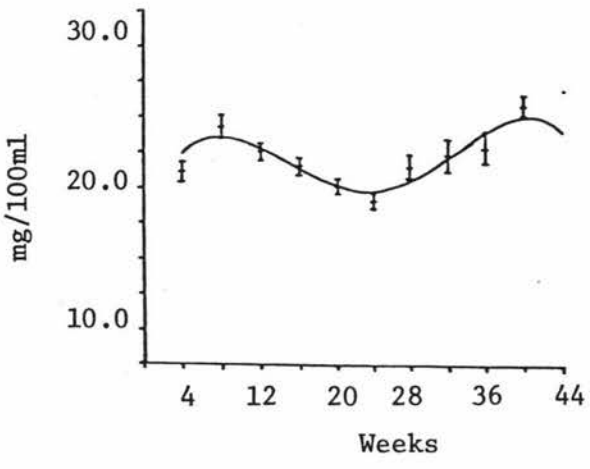


Figure IV:85

Massey No. 1 dairy unit
Spring calving group
Urea nitrogen
Weeks in milk

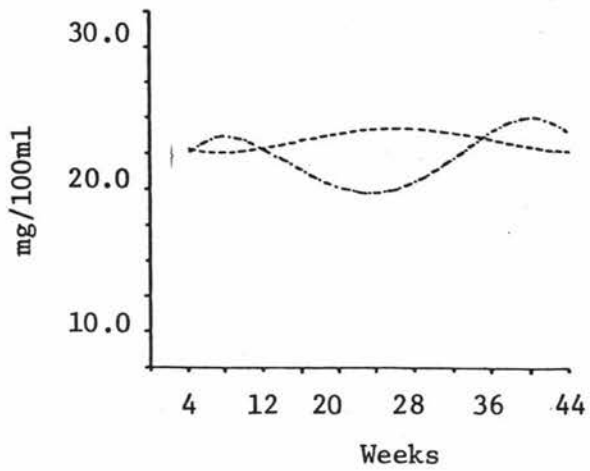


Figure IV:86

Massey No. 1 dairy unit
Autumn & spring calving grou
Urea nitrogen
Weeks in milk
Simultaneous plot

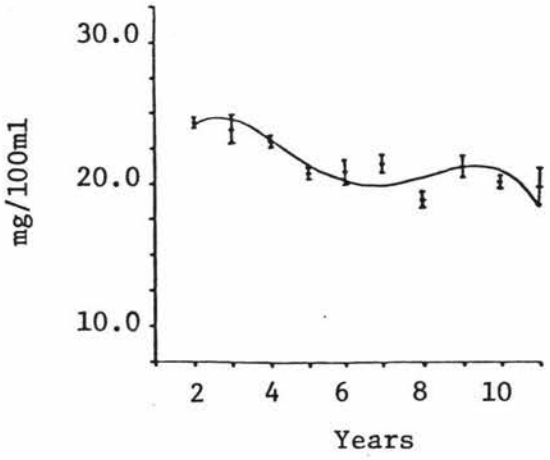


Figure IV:87

Massey No. 1 dairy unit
Urea Nitrogen
Age

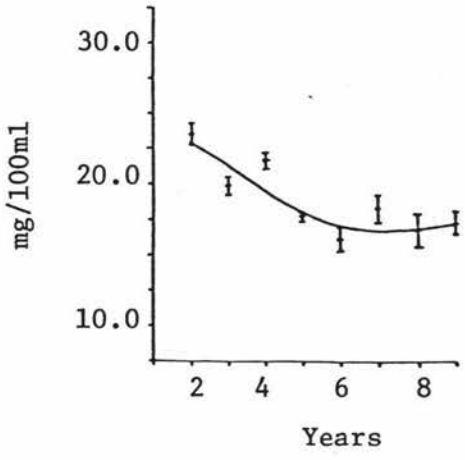


Figure IV:88

Massey No. 2 dairy unit
Urea Nitrogen
Age

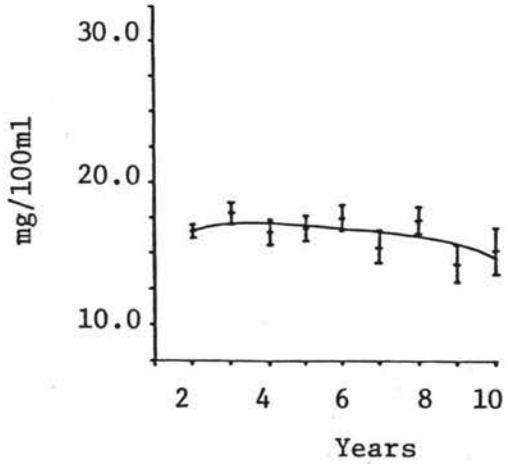


Figure IV:89

Massey No. 3 dairy unit
Urea Nitrogen
Age

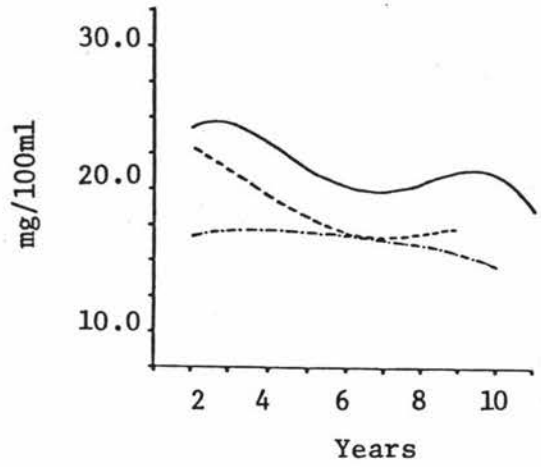


Figure IV:90

All 3 dairy units Massey
Urea Nitrogen
Age

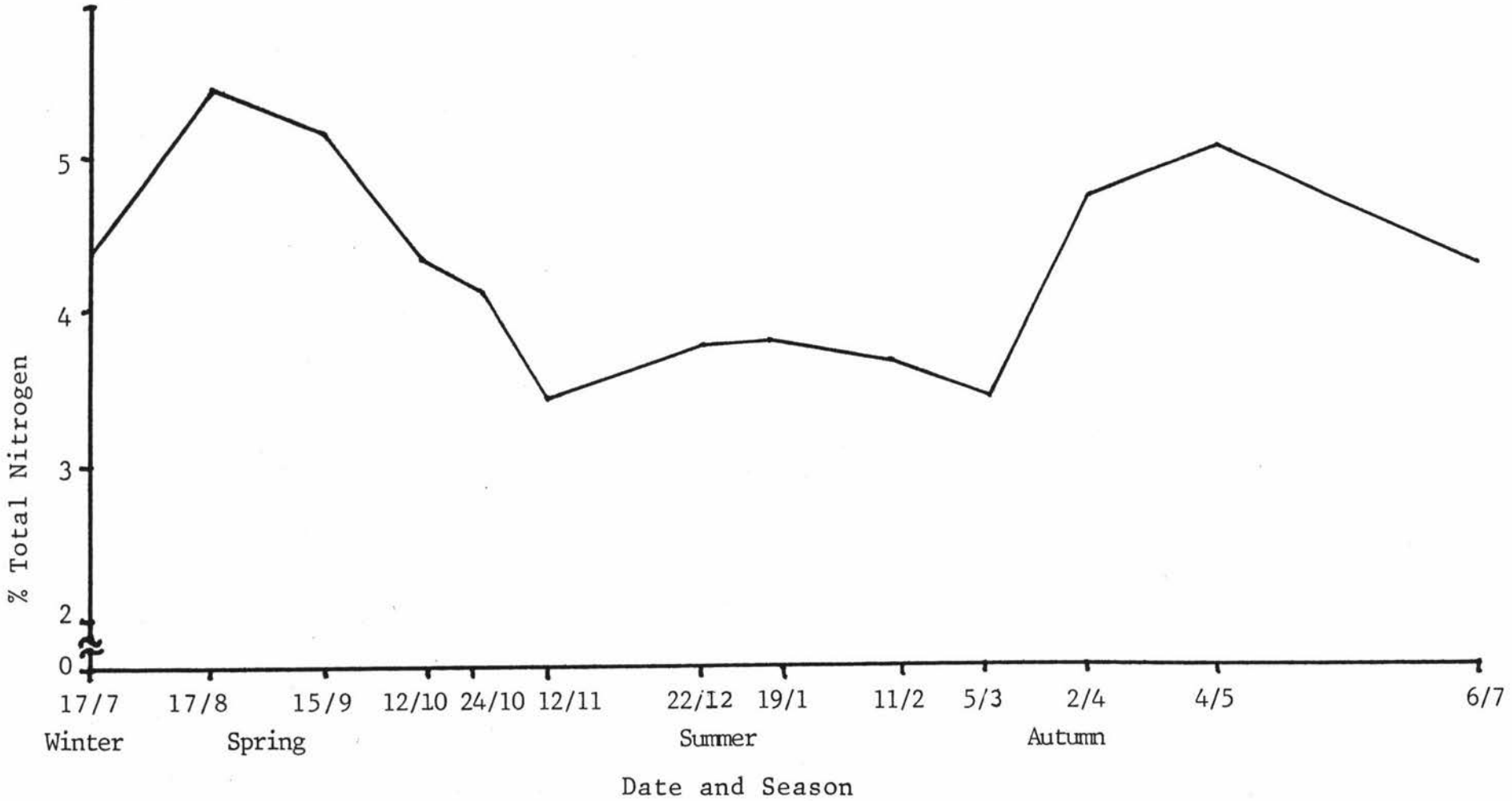


Figure IV:91; Variation in Pasture Nitrogen with Time of Year

Glucose

Direct comparison of the results for glucose with those of the Compton workers in the U.K. is difficult because of the difference in method used. They measured whole blood glucose while in this project the test was performed on plasma. A conversion table has been published (Whittard and Rose, 1971) which enables whole blood glucose values to be converted to plasma glucose levels at differing haematocrit values. In Table IV:1 the U.K. values have been converted in this manner and so can be compared with the values obtained for the Massey units. The latter in all cases are higher.

The plot for glucose value against time of year (Figs. IV:92-95) shows no consistent seasonal trend. There were lower values recorded over the summer which could have been due to the effect of temperature as reported by both Riek and Lee (1948) and Brody (1949); both sets of workers indicated that blood glucose was depressed by a high ambient temperature. The magnitude of the seasonal temperature change seen in New Zealand was not as great as that recorded in their studies which may account for the relatively inconsistent response seen on the Massey units.

Another factor affecting glucose levels, particularly as far as Unit 3 is concerned, could be level of feeding. Several authors have reported that a constant higher level of feeding results in a higher blood glucose level (see Literature Review). On Unit 3 the cows were on four different grazing systems involving two different levels of grazing intensity (1.5 and 2.0 cows per acre). This is a relatively high grazing density with strong competition between animals for feed; furthermore during this trial a drought occurred with the result that food supplies rapidly diminished after the spring flush had occurred. This fall in available food coincided with decreasing levels of blood glucose (Fig. IV:94).

Commencing mid February (between 32 and 36 weeks on Fig. IV:94), silage feeding at the rate of 5 kg dry matter (DM) per day for each cow was undertaken. Four weeks later, when the silage supply was exhausted, good quality hay was fed at the same rate for eight weeks until the cows were dried off - the supplement was then discontinued. The point to note is that although the plot is rising at the mid point of the silage feeding, the mean is altered only slightly from the prefeeding mean (50.4 mg/100ml at 32 weeks to 50.9 mg/100ml at 36 weeks - Fig. IV:94). Four weeks later, during the hay supplementation, it had risen to 60.6 mg/100ml and continued to rise during hay supplementation to 63.3 mg/100ml before falling to 57.9 mg/100ml when the hay was discontinued (Fig. IV:94). Even though the digestibility of hay and silage is similar it is possible that the reduced rate of digestion of silage relative to hay as indicated by a longer stay in the rumen (Campling, 1966), resulted in a lower rate of production of the volatile fatty acids from the structural carbohydrate components of the plants, thus accounting for a lower glucose response to the silage. The other units were not so seriuosly affected as Unit 3 as paddocks in both Unit 1 and Unit 2 were heavily irrigated. The percentage of variation explained by season is much higher for Unit 3 than for Units 1 and 2 (Table IV:2).

The plots for glucose values against weeks in milk (Figs. IV:96-99) show little similarity between units. The curves are of differing shapes and in the case of Unit 3 show similar changes to those for time of year (Fig. IV:94).

When the plot for glucose against weeks from the start was examined for the separate spring and autumn calving groups (Figs. IV:100-102) it could be seen that the glucose values at the start of the plot were marginally higher for the autumn calving group. This could be interpreted as depression of the spring calving group glucose level by the demands of lactation consistent with the statements of Hewett (1974). Later in the season, this same effect may have reduced the blood glucose values for the autumn calving group

relative to the spring calving group as the curves were seen to follow such a trend.

The plot for glucose against weeks in milk for the autumn calving group on Unit 1 (Fig. IV:103) showed a peak in plasma glucose values which was not present in the spring calving group (Fig. IV:104). The cause of this peak could be explained by the use of autumn saved pasture in mid-winter.

Generally the relationship observed between plasma glucose level and milk production was poor and it appeared that any relationship that did exist could be masked by the quantity of food being offered and consumed. Under an all grass grazing system this can be very variable because of such factors as season, fertilizer practices, calving spread and grazing management.

The indecisive relationship found in this study between glucose levels and milk production indicates why confusion exists in the literature. For instance Kronfeld (1972) claimed there was a good correlation between glucose and milk production (a high glucose level permitting the cow to sustain a high lactose production) while Hewett (1974) claimed the opposite. If a simple positive relationship existed between blood glucose levels and milk production, and the values observed for glucose on the Massey units represented an accurate estimate for the New Zealand herd, milk production should be higher than that recorded for cattle in the U.K.

Production per animal is however, lower in New Zealand than in the United Kingdom (2279 litres (Anon 1973/74a) compared with 3271 litres in the U.K. (Anon 1973/74b)). The relationship between glucose and milk production would appear to be more complex than Kronfeld (1972) suggests.

The plots for plasma glucose against age (Figs. IV:106-109) showed no consistent pattern. Probably factors such as food quantity and quality, as well as the ability to ingest and process this food, are more important than age effects *per se*.

For example the fall in glucose levels in the younger age groups in both Units 1 and 2 may have been associated with the loss of temporary incisor teeth. A number of animals were noted with infective processes involving the gums at the point of eruption of the permanent incisor teeth. At the other end of the scale the fall in glucose levels in older cattle at Unit 3 could have been a sequel to excessive teeth wear. Two older cows were culled during the sampling period and a further two at the end for this reason - they had little more than stumps level with the gums, a feature not observed on the other units.

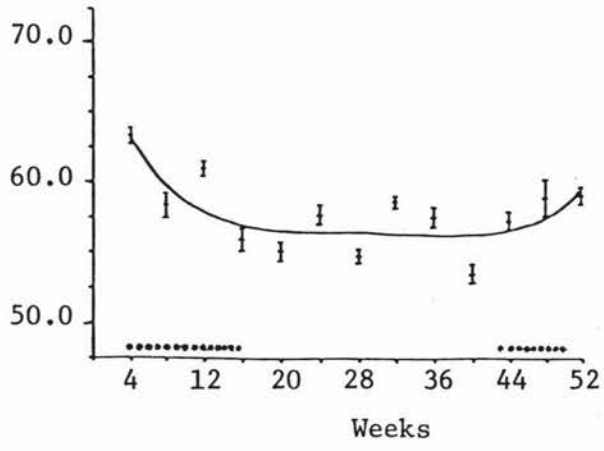


Figure IV:92

Massey No. 1 dairy unit
Glucose
Time in 4 week intervals

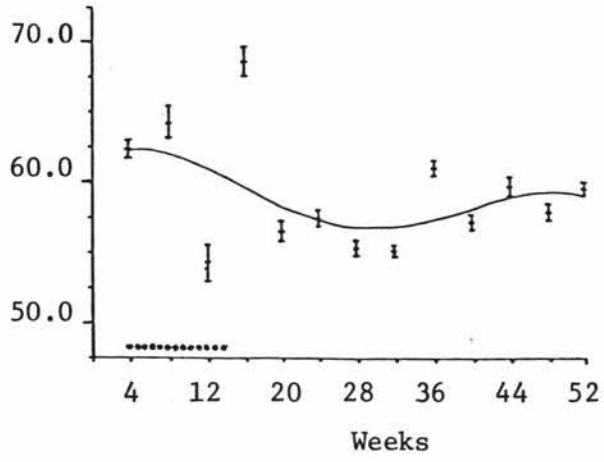


Figure IV:93

Massey No. 2 dairy unit
Glucose
Time in 4 week intervals

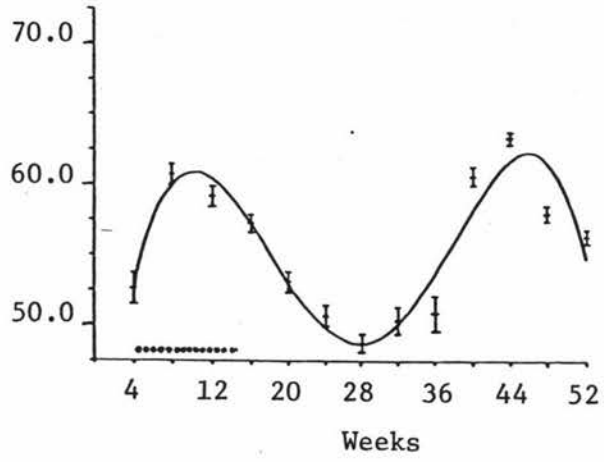


Figure IV:94

Massey No. 3 dairy unit
Glucose
Time in 4 week intervals

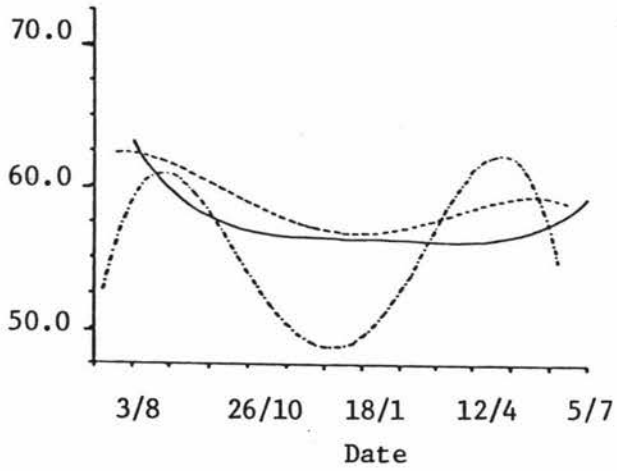


Figure IV:95

All 3 dairy units Massey
Glucose
Simultaneous plot

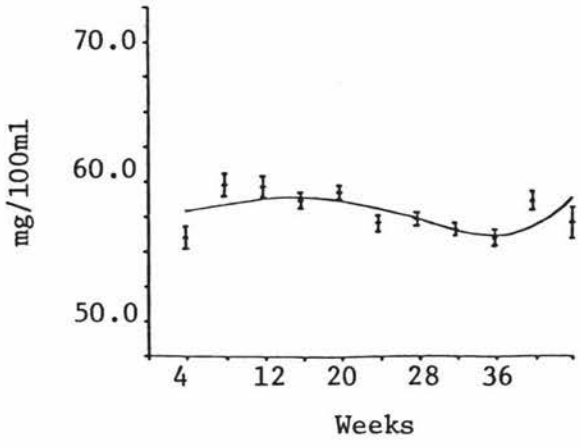


Figure IV:96

Massey No. 1 dairy unit
Glucose
Weeks in milk

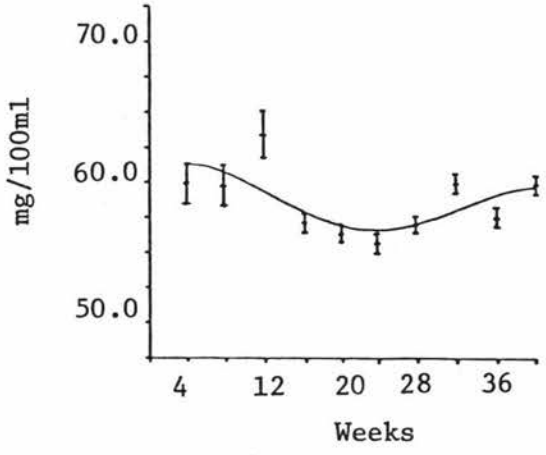


Figure IV:97

Massey No. 2 dairy unit
Glucose
Weeks in milk

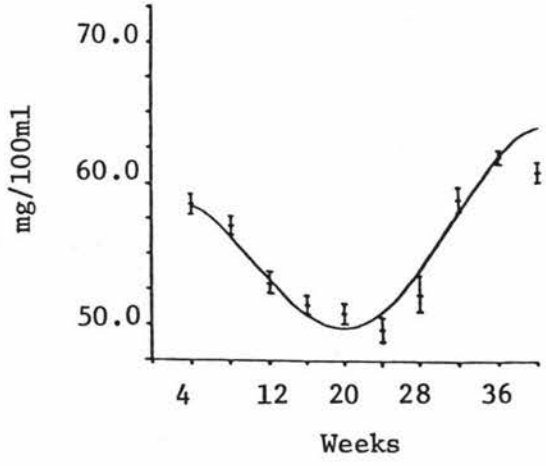


Figure IV:98

Massey No. 3 dairy unit
Glucose
Weeks in milk

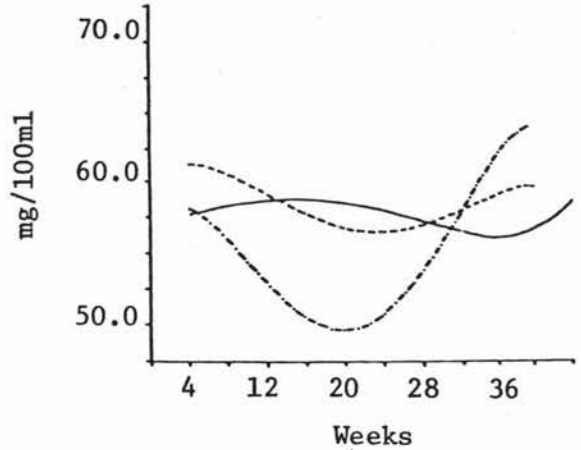


Figure IV:99

All 3 dairy units Massey
Glucose
Weeks in milk

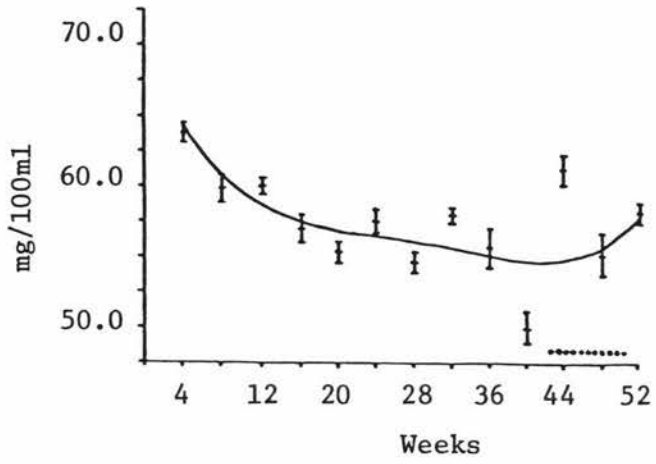


Figure IV:100

Massey No. 1 dairy unit
Autumn calving group
Glucose
Time in 4 week intervals

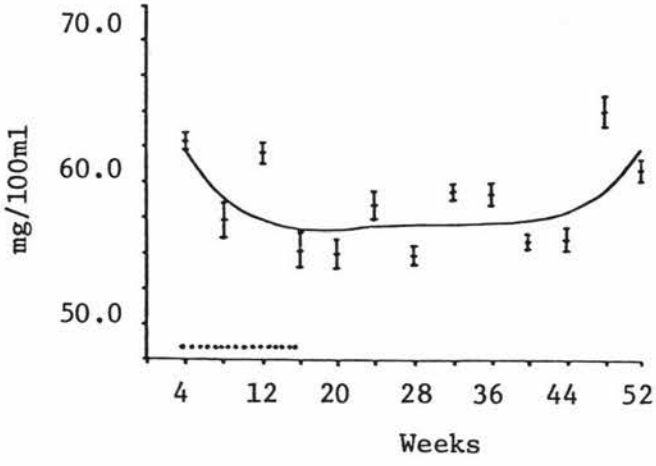


Figure IV:101

Massey No. 1 dairy unit
Spring calving group
Glucose
Time in 4 week intervals

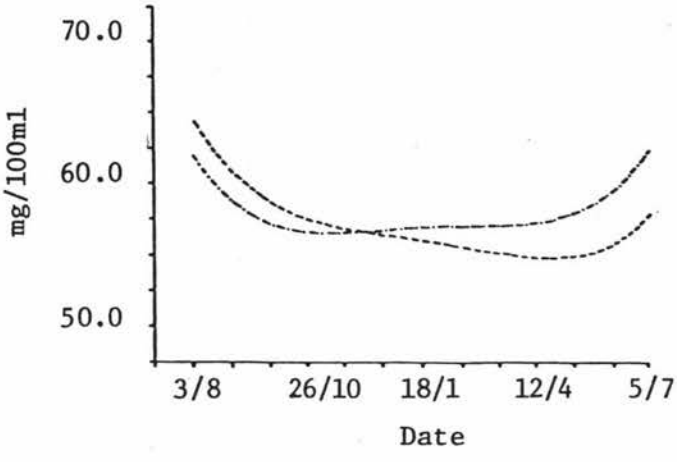


Figure IV:102

Massey No. 1 dairy unit
Autumn & spring calving groups
Glucose
Time in 4 week intervals
Simultaneous plot

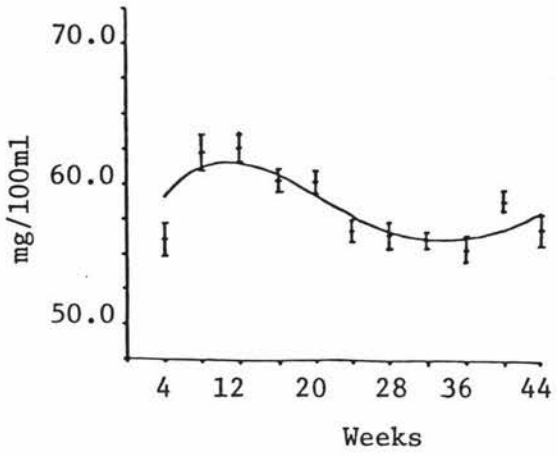


Figure IV:103

Massey No. 1 dairy unit
Autumn calving group
Glucose
Weeks in milk

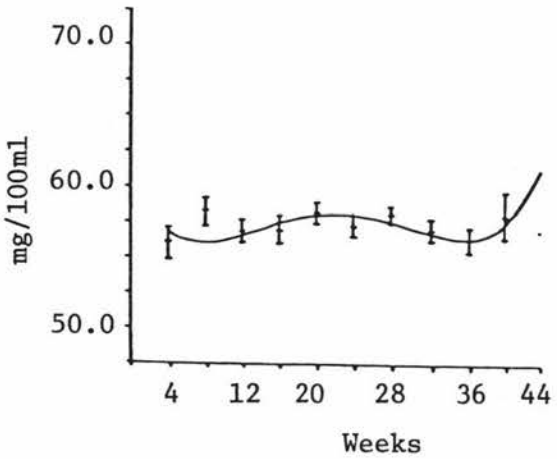


Figure IV:104

Massey No. 1 dairy unit
Spring calving group
Glucose
Weeks in milk

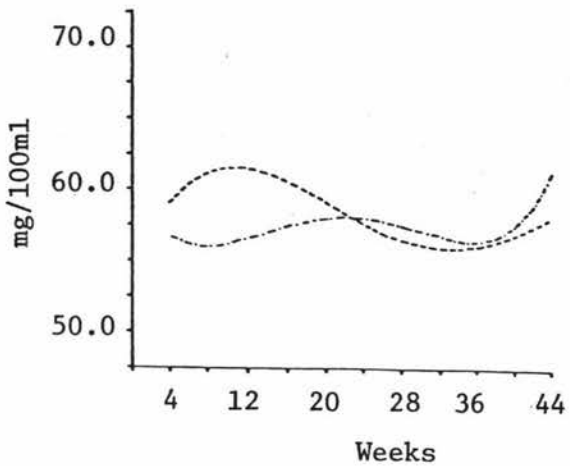


Figure IV:105

Massey No. 1 dairy unit
Autumn & spring calving groups
Glucose
Weeks in milk
Simultaneous plot

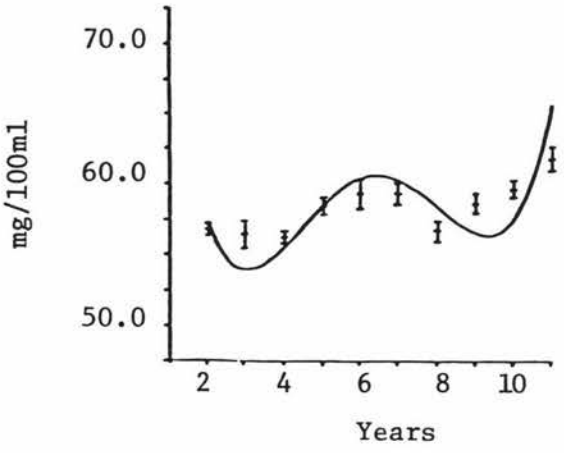


Figure IV:106

Massey No. 1 dairy unit
Glucose
Age

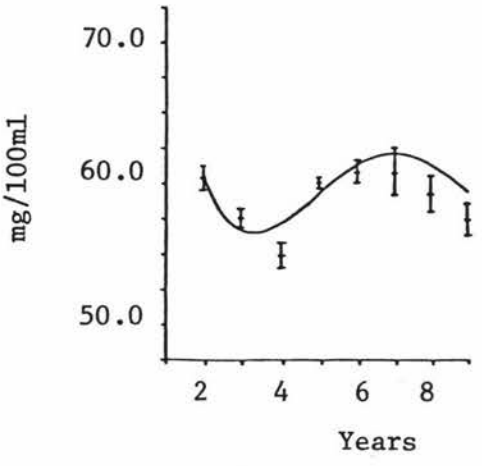


Figure IV:107

Massey No. 2 dairy unit
Glucose
Age

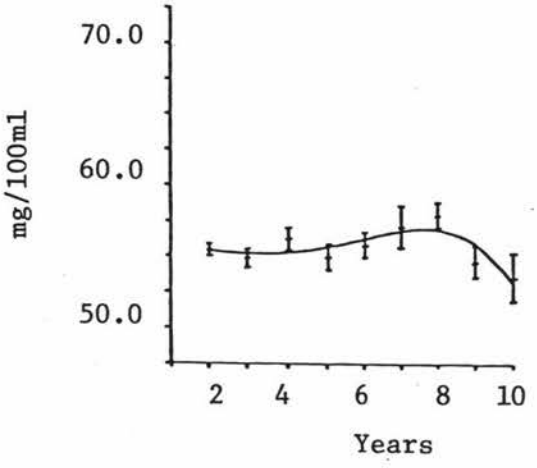


Figure IV:108

Massey No. 3 dairy unit
Glucose
Age

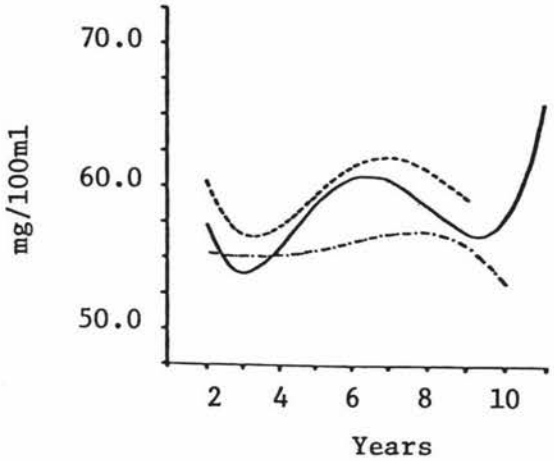


Figure IV:109

All 3 dairy units Massey
Glucose
Age

Sodium and Potassium

These two elements are considered together since, as the major cations of extracellular and intracellular fluids respectively, modulation of membrane function results in reciprocal changes in their concentrations. In health, the homeostatic controls of both cations is such that plasma concentrations are held within relatively narrow limits (Payne *et al.*, 1974; Rowlands *et al.*, 1974). This is clearly illustrated by the small standard deviation about the mean reported in the literature.

Serum concentrations for sodium and potassium for herds on the Massey properties are summarised in Table IV:1. In all cases both means and standard deviations for sodium are considerably higher than the values reported for the U.K. The mean is in fact more than two standard deviations above the U.K. mean. The question arises as to whether this is a real difference or whether there is some other explanation for the variation in results that has been obtained.

Sample evaporation leading to increases in the concentration of sodium, and errors in preparation of the standards (which should not increase the standard deviation) do not provide satisfactory explanations for the differences observed and the problem appears to rest with the variation in results obtained with the analytical method used.

The assay for sodium involves flame photometric assay of serum dialysate (see Material and Methods p. 141). To allow correction for variation due to the instrumentation the method indicates that a standard be included every ten samples and that adjustments be made to sample values according to the variations in the readings of the standard. From the start of the project marked variations were experienced in the readings of the standards for sodium and adjustments were made until the correction factor became too large and complete recalibration of the machine necessary. As a

consequence of this variation, and the often large corrections necessary, inaccuracies remained in the data reported.

The probable cause of the shifting sensitivity was associated with environmental temperature fluctuations; the laboratory where the analysis was done was quite a small room and virtually by accident, it was noted that temperature increases due to operation of the flame photometer could be overcome when the door was left open. Thus during the next part of the project the test was run with the door and window wide open. This resulted in a more stable temperature, a greater stability of standard readings, and more realistic values for serum sodium. Unfortunately this was not appreciated when samples during Part A were being run and it is probable that the values obtained in this part of the work were unreliable estimates.

The means and standard deviations for potassium during Part A of the project were higher than the U.K. figures by the same proportion as sodium was higher than the U.K. mean (Table IV:1). It is probable therefore that the same variability which occurred in the sodium results also occurred with potassium.

On examination of the figures for bleed by season (Figs. IV: 110-113) the particularly low value for sodium for Unit 1 at the initial bleed requires comment. The mean value obtained is in error and associated with the problems in technique at this time. Reference back to the original observations for this run shows that there were two groups of samples, those with a mean value above 134 mEq/l and those with a value below 124 mEq/l. Survival of the animal would be unlikely at this lower figure. If this initial sample for Unit 1 is ignored the curve is similar to the curve obtained for the other units.

Examining all the units together, the main change in sodium value seems to be a rise from a relatively low point at the start of the season, which continues over the summer and then falls during the following autumn and winter. This is at variance with the figures reported by Payne *et al.* (1972b, 1973, 1974) and Rowlands *et al.* (1974), all of whom report a drop in summer and a rise in winter. As the majority of cows in the report by Rowlands *et al.* (1974) calved in autumn, and therefore were at peak lactation when the peak of sodium occurred, their finding is somewhat surprising, since a high intake of the element would be required to counter the losses in milk (Payne *et al.*, 1972b). A high sodium supplementation rate in the diet of cattle in the U.K. may account for this. If on the other hand a drop in sodium level is expected at peak lactation, this would account for the low values obtained with the first two or three samples following calving in the two seasonal supply units at Massey.

The rise in sodium levels on all Massey units during the summer months (Figs. IV:110-113) could be due to a variety of factors. Evaporation of the sample in a warmer environment could have occurred to some extent in spite of precautions taken to minimise this effect. Another factor that could have influenced the sodium level was water deprivation, especially on Unit 3. Water supply lagged behind requirement at this time of year and the water troughs were observed to be empty early in the day during the grazing period with the cattle then drinking the water as it flowed into the trough. If this did result in dehydration it could account for an elevation of serum sodium over this time period.

An interesting feature of the sodium plots was the fall that occurred near the end of lactation (Figs. IV:114-117). The plot for potassium against stage of lactation (Figs. IV:132-135) revealed a concurrent rise in the case of Units 2 and 3. Examination of the means and standard deviations for these

two elements shows that even though the fitted graph tends to accentuate the changes, they are nevertheless real. Dietary imbalance of excessive potassium and inadequate sodium has been recorded in cattle on a forage diet (Payne *et al.*, 1972b) and this is one possible explanation for this occurring. No effect of declining level of lactation on potassium values has been reported.

Referring to Table IV:2 it appears that seasonal influences play a significant part in the variation that is recorded on Unit 1 but much less so for the other two units. The validity of the result for Unit 1 must be in doubt however, because of the influence of the first set of sample values on the data discussed earlier.

When the plots for effect of lactation on sodium levels of the different units are examined (Figs. IV:114-117), the same reproducible pattern emerges, although the effects are somewhat exaggerated in the later stages of lactation for Unit 3. All units show a rise through the first two-thirds of lactation. Whether this is a lactational or seasonal effect cannot be determined as each accounts for a similar portion of the variation.

As with the haemoglobin and haematocrit curves, the relationship of sodium levels with stage of lactation for the separate spring and autumn herds on Unit 1 (Figs. IV:121-123) show a difference in phase. When shifted relative to each other so that time of year coincides (Fig. IV:120) the fit is much closer indicating that the seasonal influence is more pronounced than that due to lactation. The percentage of variation in sodium values explained by season is much greater than that explained by lactation (Table IV:2).

Age influences on sodium levels were significant only for Unit 1 where they explained only 0.2% of the variation (Table IV:2). Because of the importance of sodium as an osmo-regulator marked changes in serum level with age would not be expected.

When the plots for potassium against weeks from the start of observations are studied (Figs. IV:128-131) little change in absolute terms is revealed in the graph for potassium. The peak of the curve for Unit 1 occurs in the autumn when the feed is relatively dry and the plant extracellular fluid low; at this stage the feed could be expected to have a low sodium:potassium ratio (Payne *et al.*, 1972b). Why such an effect was not seen on the other units is not known.

The graphs for potassium against weeks in milk for the separate spring and autumn calving groups on Unit 1 (Figs. IV:139-141) exhibit the same phase differences as were commented on in the discussion of sodium. When the curves are adjusted so that the time of year coincides the graph closely resembles the plot of potassium against weeks from the start for this Unit (Fig. IV:128). This suggests that season is a more important source of variation than lactation, an observation which agrees with the results in Table IV:2.

The plots for potassium against weeks in milk (Figs. IV:132-135) do not appear to represent any change which is consistent for all three units. A prominent feature in the curve drawn for Unit 3 (Fig. IV:134) is the sharp rise at the end of lactation. The high values which cause this arise from an artefact. The mean level of potassium achieved in the analysis for sample week 44 was abnormally high and a number of individual values were not compatible with continued life. The terminal rise should therefore be disregarded.

The plots for potassium against weeks from the start for the spring and autumn calving groups on Unit 1 (Figs. IV:136-138), which should illustrate any seasonal differences that exist in two groups of animals at different stages of lactation, did not reveal any obvious lactational effect even though 11.5% of the variation observed was accounted for by this source (Table IV:2).

No consistent age effects on potassium were observed on any of the units (Figs. IV:142-145).

The evidence indicated that the nature of the changes in these two elements were basically similar in form. If the changes were the result of variations in the rate of mineralocorticoid secretion, movements in an inverse fashion would have been the case. As this did not occur it is likely that these variations are the result of other factors such as nutrition and lactation.

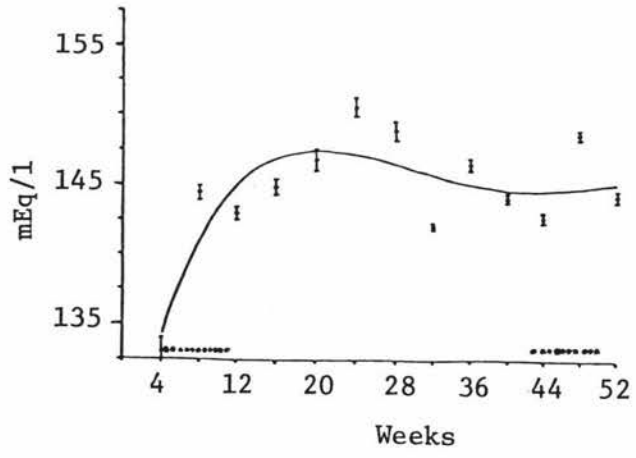


Figure IV: 110

Massey No. 1 dairy unit
Sodium
Time in 4 week intervals

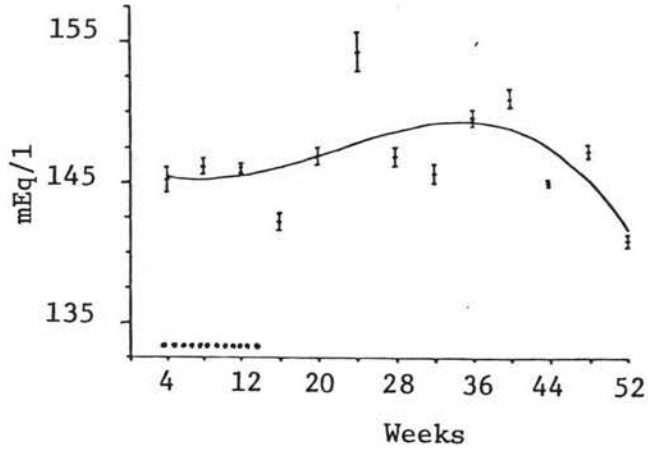


Figure IV: 111

Massey No. 2 dairy unit
Sodium
Time in 4 week intervals

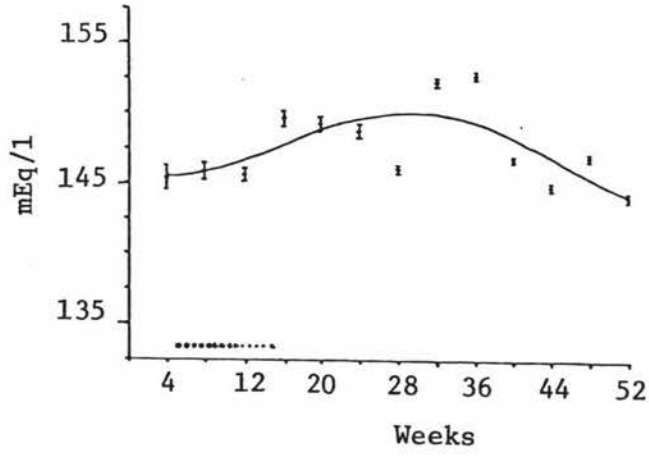


Figure IV: 112

Massey No. 3 dairy unit
Sodium
Time in 4 week intervals

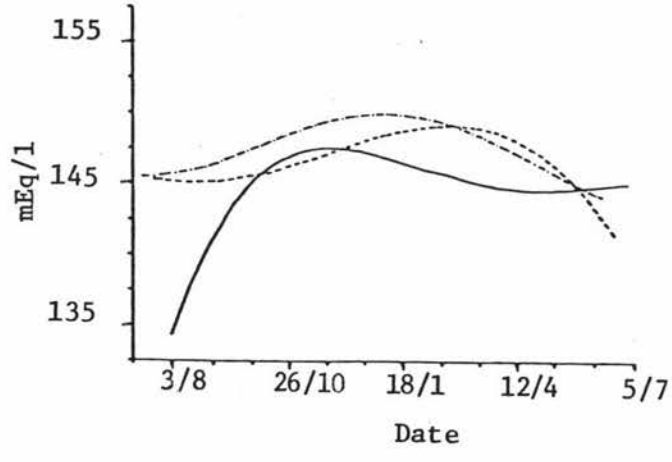


Figure IV: 113

All 3 dairy units Massey
Sodium
Simultaneous plot

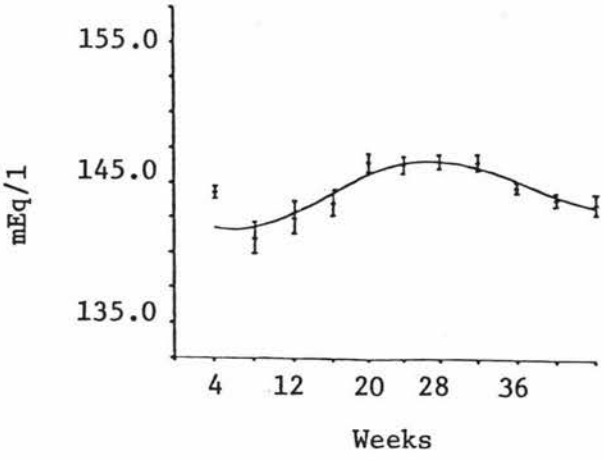


Figure IV: 114

Massey No. 1 dairy unit
Sodium
Weeks in milk

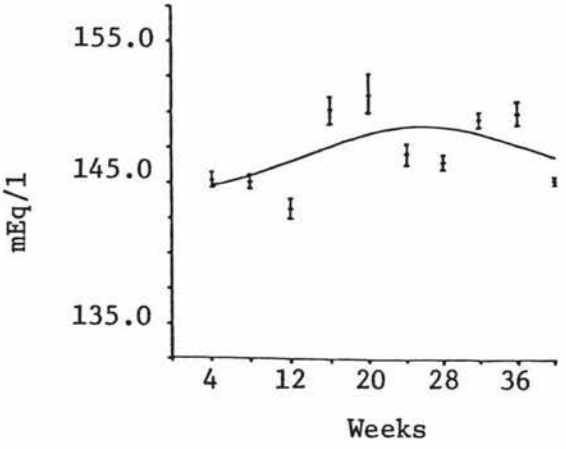


Figure IV: 115

Massey No. 2 dairy unit
Sodium
Weeks in milk

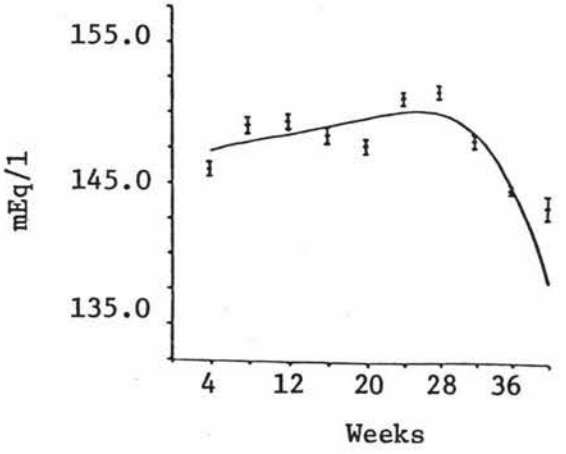


Figure IV: 116

Massey No. 3 dairy unit
Sodium
Weeks in milk

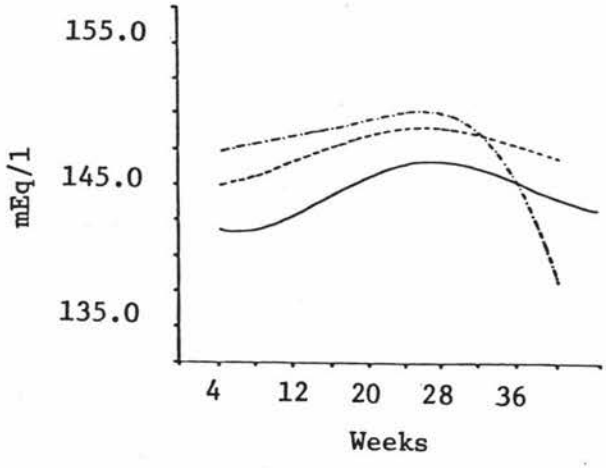


Figure IV: 117

All 3 dairy units Massey
Sodium
Weeks in milk

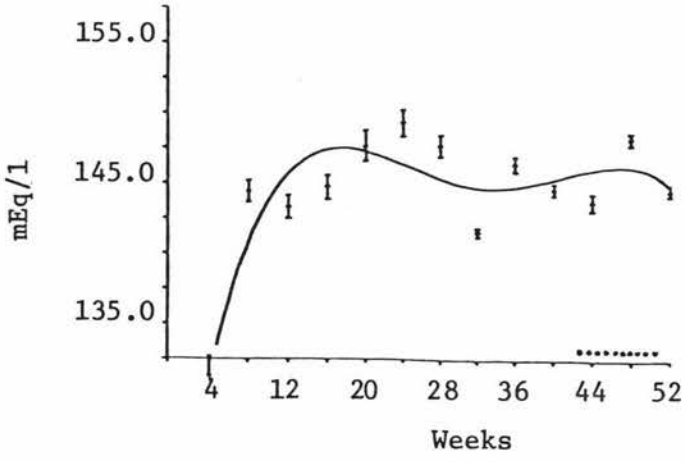


Figure IV: 118

Massey No. 1 dairy unit
Autumn calving group
Sodium
Time in 4 week intervals

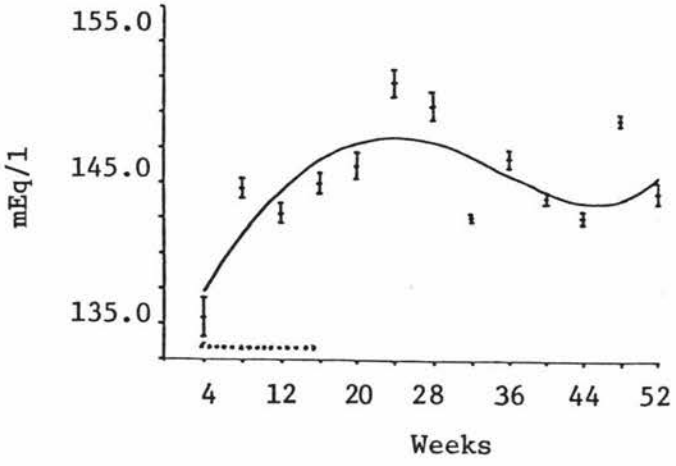


Figure IV: 119

Massey No. 1 dairy unit
Spring calving group
Sodium
Time in 4 week intervals

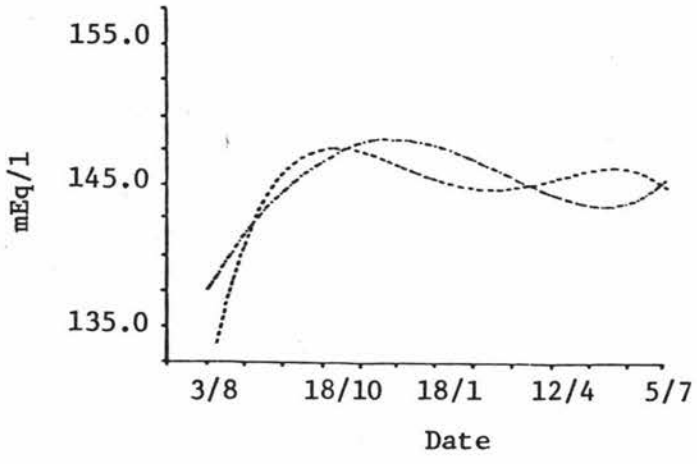


Figure IV: 120

Massey No. 1 dairy unit
Autumn & spring calving groups
Sodium
Time in 4 week intervals
Simultaneous plot

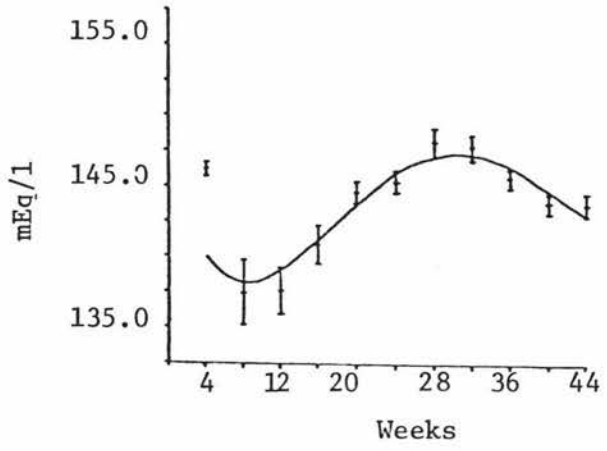


Figure IV: 121

Massey No. 1 dairy unit
Autumn calving group
Sodium
Weeks in milk

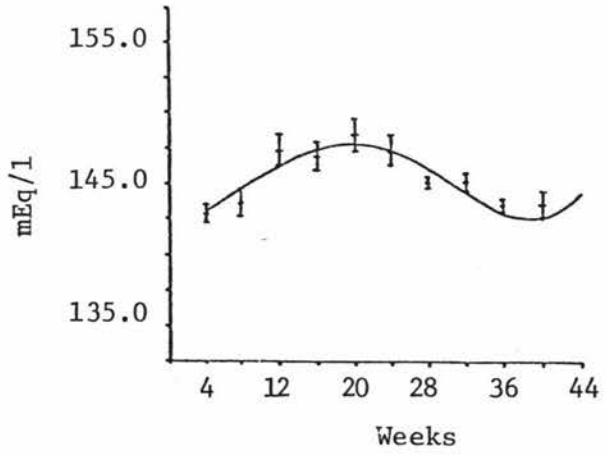


Figure IV: 122

Massey No. 1 dairy unit
Spring calving group
Sodium
Weeks in milk

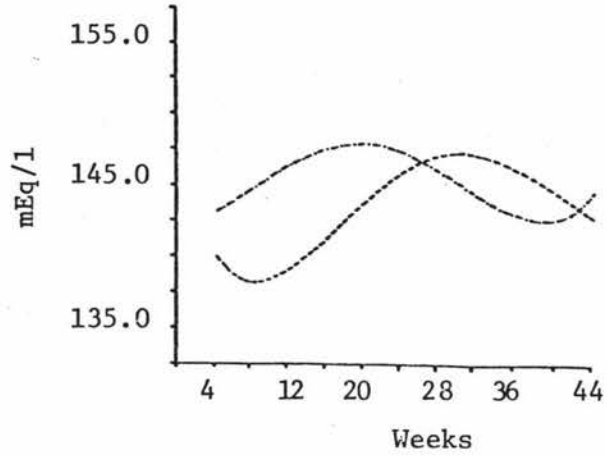


Figure IV: 123

Massey No.1 dairy unit
Autumn & spring calving group
Sodium
Weeks in milk
Simultaneous plot

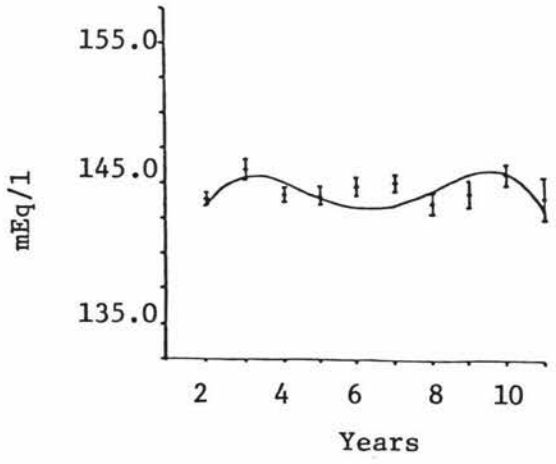


Figure IV: 124

Massey No. 1 dairy unit
Sodium
Age

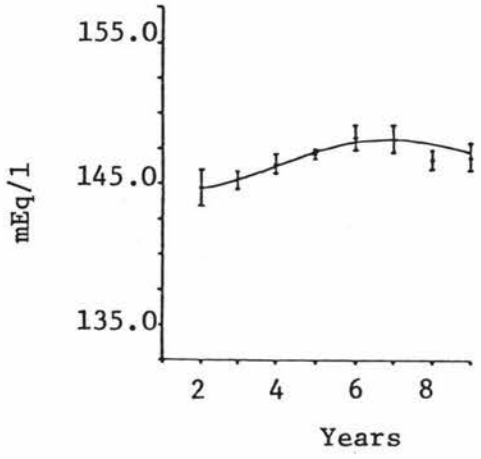


Figure IV: 125

Massey No. 2 dairy unit
Sodium
Age

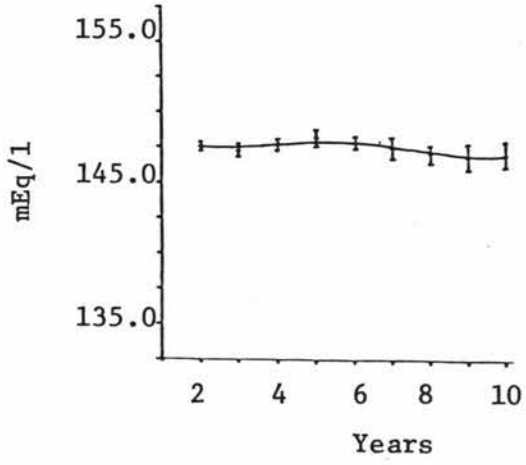


Figure IV: 126

Massey No. 3 dairy unit
Sodium
Age

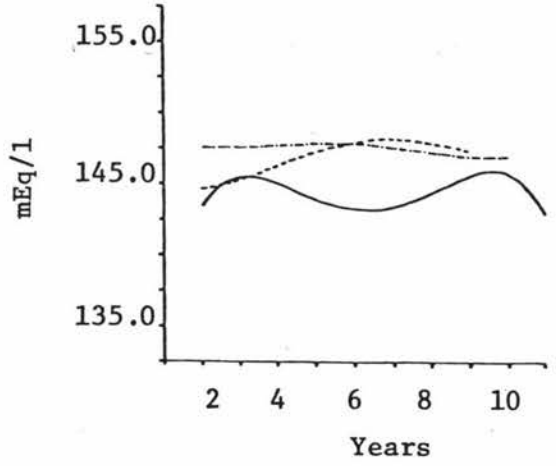


Figure IV: 127

All 3 dairy units Massey
Sodium
Age

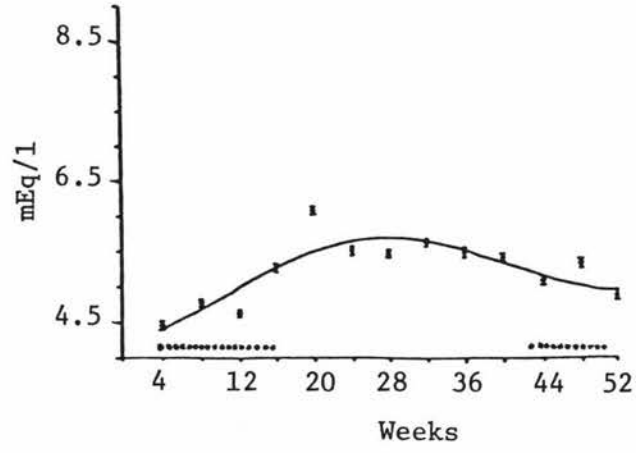


Figure IV: 128

Massey No. 1 dairy unit
Potassium
Time in 4 week intervals

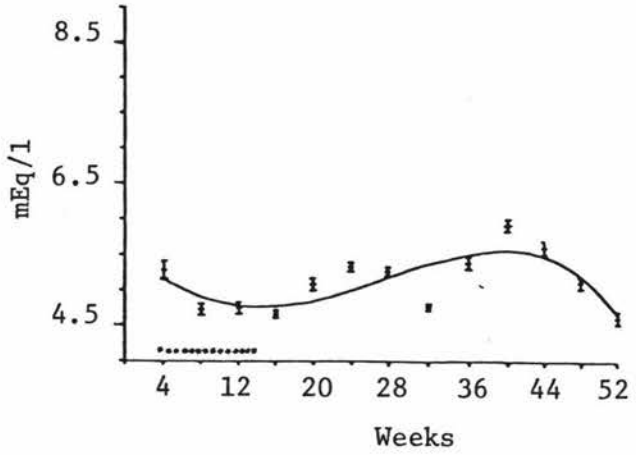


Figure IV: 129

Massey No. 2 dairy unit
Potassium
Time in 4 week intervals

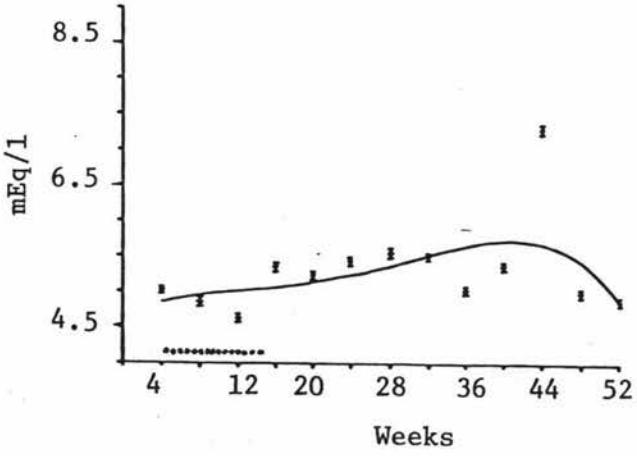


Figure IV: 130

Massey No. 3 dairy unit
Potassium
Time in 4 week intervals

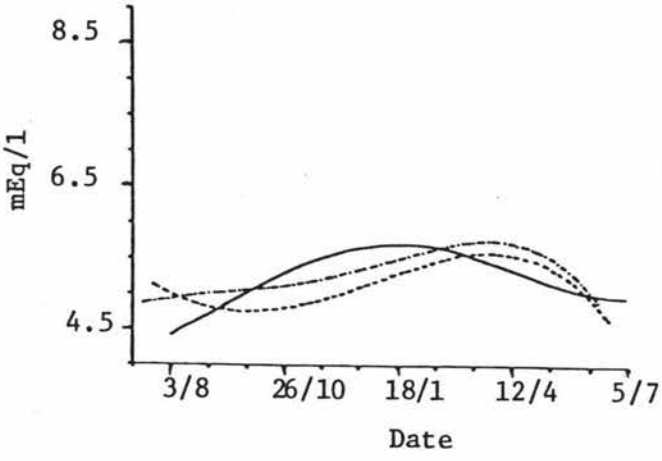


Figure IV: 131

All 3 dairy units Massey
Potassium
Simultaneous plot

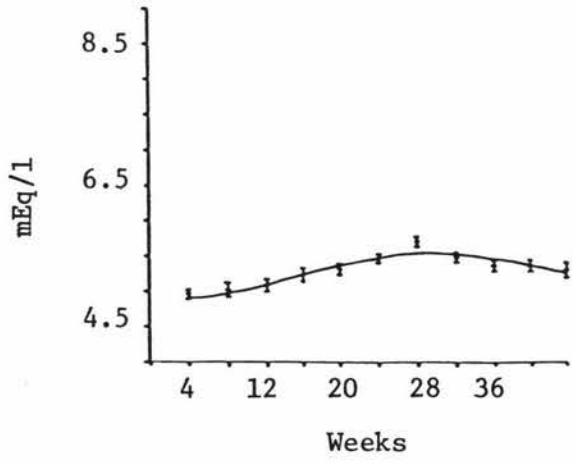


Figure IV: 132

Massey No. 1 dairy unit
Potassium
Weeks in milk

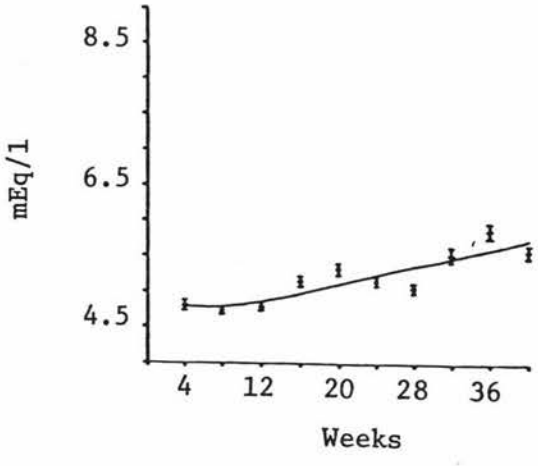


Figure IV: 133

Massey No. 2 dairy unit
Potassium
Weeks in milk

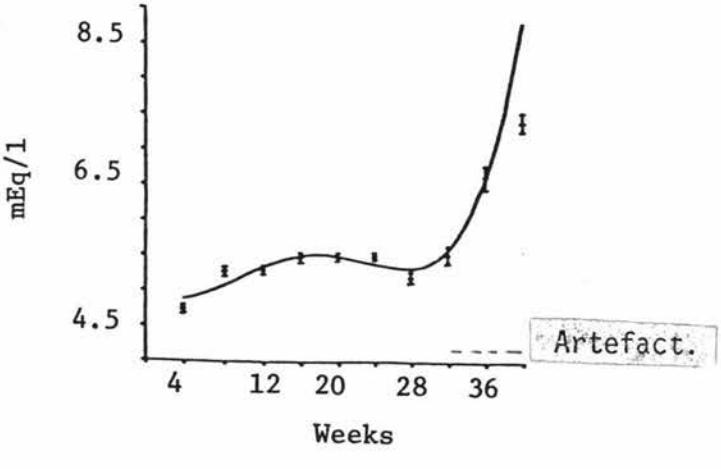


Figure IV: 134

Massey No. 3 dairy unit
Potassium
Weeks in milk

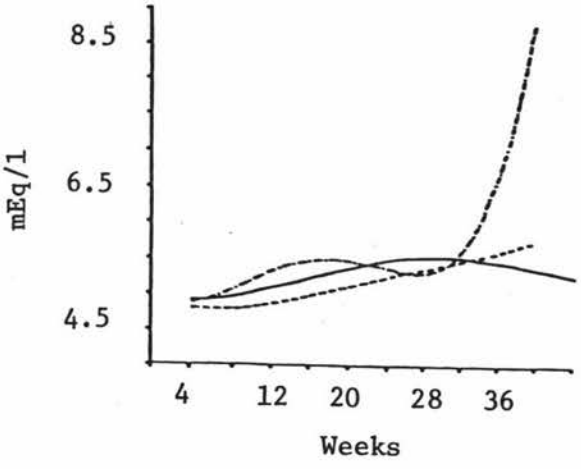


Figure IV: 135

All 3 dairy units Massey
Potassium
Weeks in milk

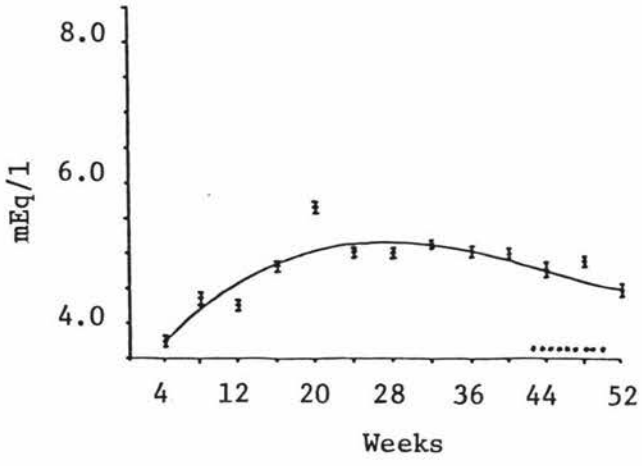


Figure IV: 136

Massey No. 1 dairy unit
Autumn calving group
Potassium
Time in 4 week intervals

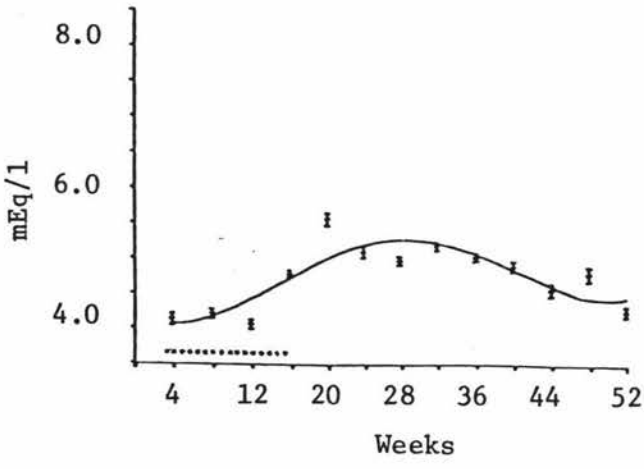


Figure IV: 137

Massey No. 1 dairy unit
Spring calving group
Potassium
Time in 4 week intervals

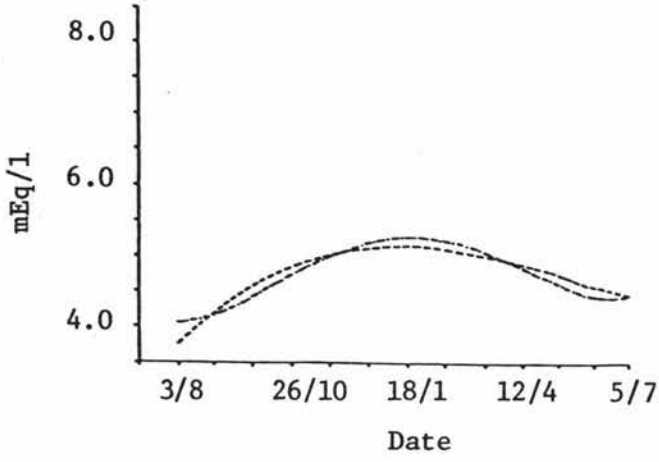


Figure IV: 138

Massey No. 1 dairy unit
Autumn & spring calving groups
Potassium
Time in 4 week intervals
Simultaneous plot

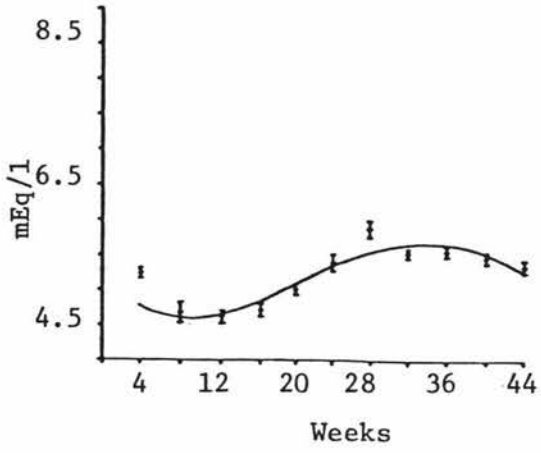


Figure IV: 139

Massey No. 1 dairy unit
Autumn calving group
Potassium
Weeks in milk

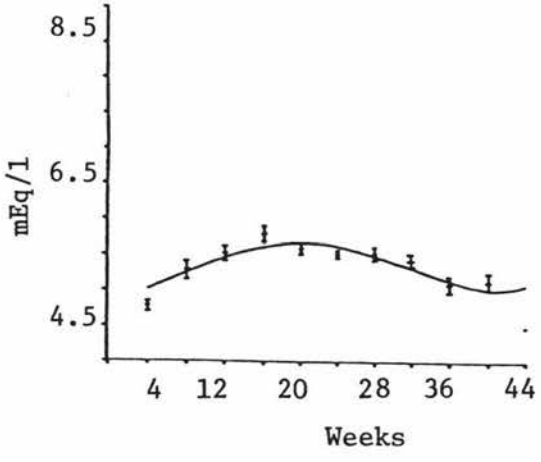


Figure IV: 140

Massey No. 1 dairy unit
Spring calving group
Potassium
Weeks in milk

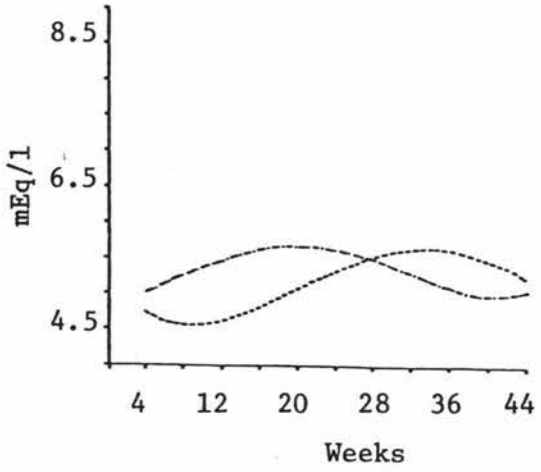


Figure IV: 141

Massey No. 1 dairy unit
Autumn & spring calving groups
Potassium
Weeks in milk
Simultaneous plot

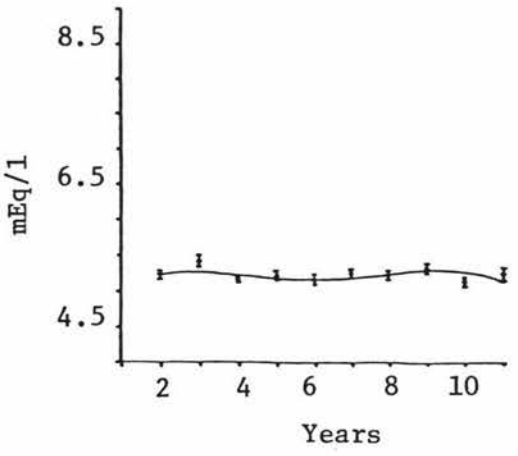


Figure IV: 142

Massey No. 1 dairy unit
Potassium
Age

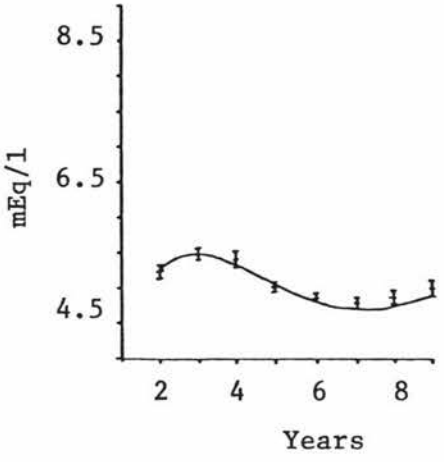


Figure IV: 143

Massey No. 2 dairy unit
Potassium
Age

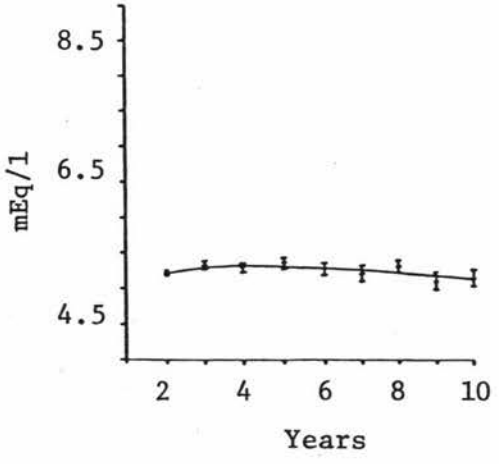


Figure IV: 144

Massey No. 3 dairy unit
Potassium
Age

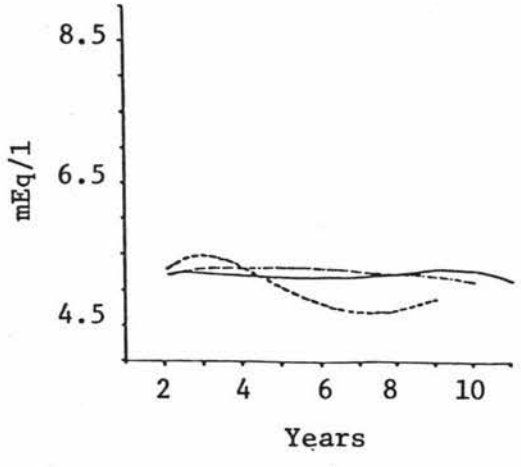


Figure IV: 145

All 3 dairy units Massey
Potassium
Age

The mean serum magnesium levels were lower on all three Massey units than are the figures reported for the U.K. (Table IV:1). Since availability rather than content of magnesium in the feed appears to be the important factor in determining how much is absorbed, and as magnesium in a forage diet is frequently of a low availability (see Review of Literature), it is not surprising that the New Zealand all year round grazing system leads to serum levels that are lower than with cattle which spend a proportion of the year housed indoors and fed a more balanced ration as in the U.K. Furthermore the supplemented cattle in the U.K. receive rations which exceed minimum requirements of magnesium according to Payne *et al.* (1970b), and this during that period of the year (winter) when in a grazing management system serum magnesium levels are at their lowest (Young *et al.*, 1979).

Excess magnesium in the diet results in a decreased rate of intestinal absorption and an increased urinary excretion as the animals homeostatic mechanisms operate to maintain serum magnesium stability (O'Kelley & Fontenot , 1969); above a certain threshold of intake variability of serum magnesium is likely to be reduced. If this is the case it is also easy to understand why the standard deviation for magnesium level in the New Zealand cattle on their more variable diet is higher than that for their U.K. counterparts (Table IV:1).

Energy intake (see Review of Literature) also has a bearing on the animals ability to absorb dietary magnesium. This may be the explanation for the wide variation of values recorded on Unit 3 (Table IV:1), a unit on which cattle were underfed for a considerable proportion of the trial period (see pp 125-6).

The plots for magnesium level against time of year (Figs. IV: 146-149) showed rather different movements for each unit. On Unit 1 the pattern was similar to that associated with the seasonal change in protein composition of the pasture (Fig. IV:91). It should be noted however that the means were not a

close fit about the curve and the amount of variation explained by season was only 8% (Table IV:2). The plots for magnesium against weeks in milk for the separate spring and autumn calving groups (Figs. IV:157-159) also tend to reflect this same pattern. The high level at about week 20 in the autumn calving group was approximately equal to that at weeks 4-8 for the spring calving group while the peak at 36 weeks for the latter group was equivalent to the rise at the end of the curve for the autumn calving group. As these corresponded with peaks of pasture growth and high protein levels they probably reflected total intake rather than altered availability since there is evidence that increased nitrogen content of the diet results in a reduction in the availability of magnesium (see Review of Literature).

The plot for magnesium against time of year for Unit 2 (Fig. IV:147) showed pronounced and significant changes at the start of the graph when magnesium levels were low, consistent with the observation of low magnesium availability and low serum magnesium levels reported elsewhere in the winter and early spring period (see Review of Literature). Following the spring growth the level of food intake improved and the serum magnesium level rose. This was followed by a fall in serum magnesium in the autumn which could be associated with either a fall in intake and/or changes to the mineral status that followed the transfer of the stock to a different area (p 124). The final stage of the plot for magnesium for this unit did not therefore necessarily reflect a true seasonal effect. That the serum magnesium level on Unit 2 was sustained over the summer period whereas on Unit 1 it fell cannot be satisfactorily explained; it could have been due to feeding differences resulting from the management associated with calving at two separate times of the year in the latter herd. It could also have been associated with management changes at the end of lactation.

The plot for magnesium against time of year for Unit 3 (Fig. IV:148) illustrated a scatter of means about an almost straight line. It is not clear why this should have been so different from the other units.

The plots for magnesium against weeks in milk (Figs. IV:150-153) showed relatively minor changes between units and the amount of variation that could be explained by the effects of lactation was low (Table IV:2). Milk production appeared to have little influence on serum magnesium levels, an observation which was reinforced by examining the plots for the separate spring and autumn calving groups on Unit 1 (Figs. IV:157-159) where the curves were clearly out of phase and reflected the changes in season.

No significant changes in serum magnesium level associated with age were observed (Figs. IV:160-163).

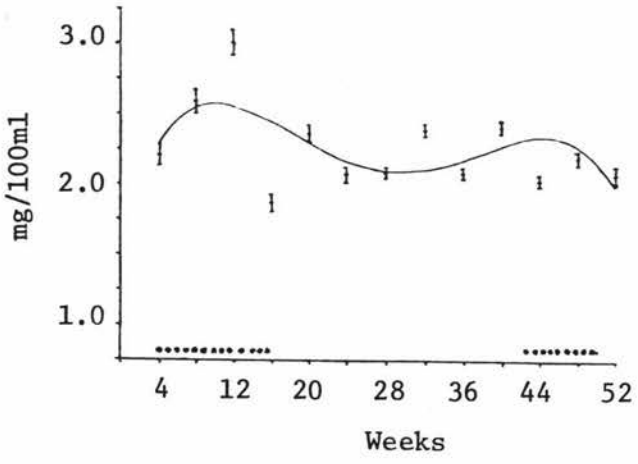


Figure IV: 146

Massey No. 1 dairy unit
Magnesium
Time in 4 week intervals

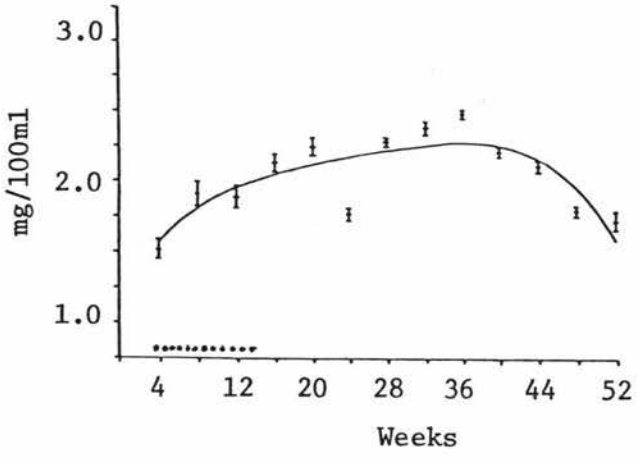


Figure IV: 147

Massey No. 2 dairy unit
Magnesium
Time in 4 week intervals

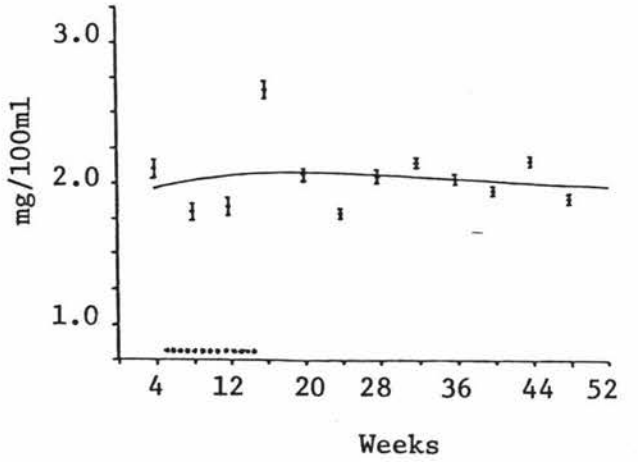


Figure IV: 148

Massey No. 3 dairy unit
Magnesium
Time in 4 week intervals

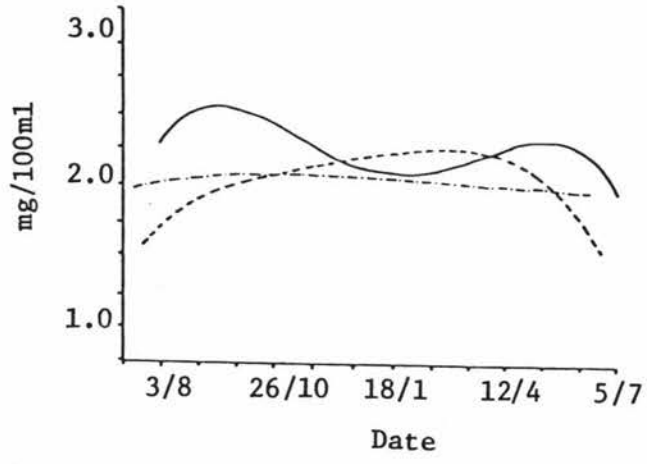


Figure IV: 149

All 3 dairy units Massey
Magnesium
Simultaneous plot

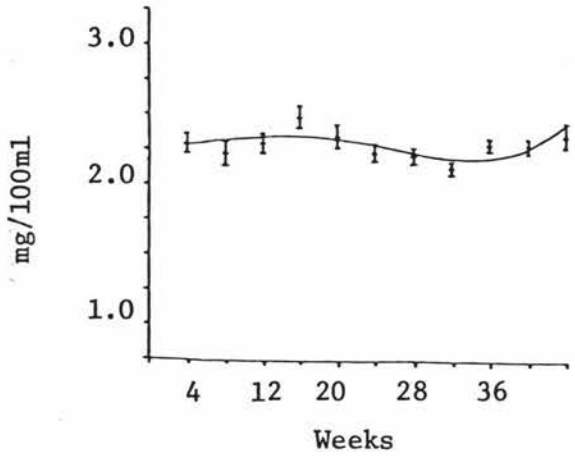


Figure IV: 150

Massey No. 1 dairy unit
Magnesium
Weeks in milk

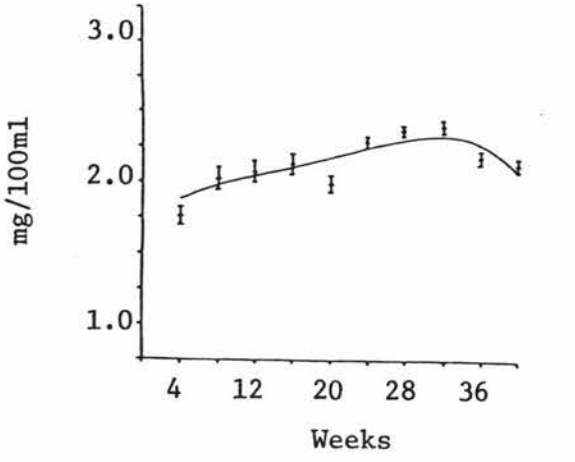


Figure IV: 151

Massey No. 2 dairy unit
Magnesium
Weeks in milk

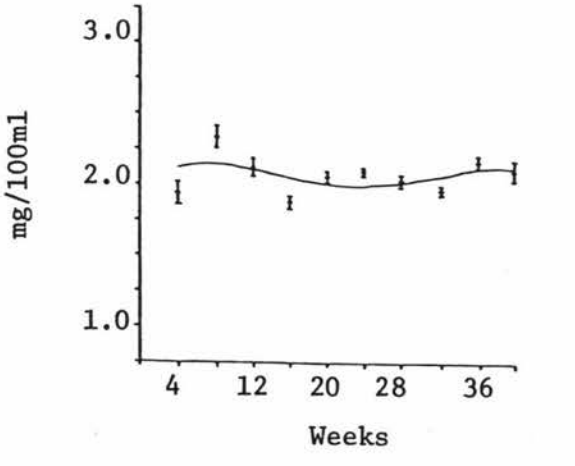


Figure IV: 152

Massey No. 3 dairy unit
Magnesium
Weeks in milk

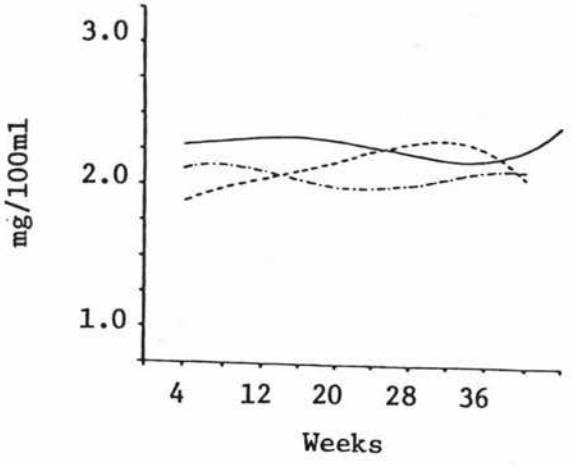


Figure IV: 153

All 3 dairy units Massey
Magnesium
Weeks in milk

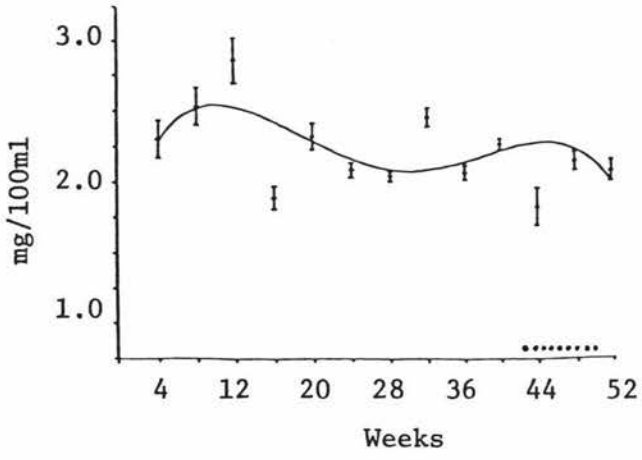


Figure IV: 154

Massey No. 1 dairy unit
Autumn calving group
Magnesium
Time in 4 week intervals

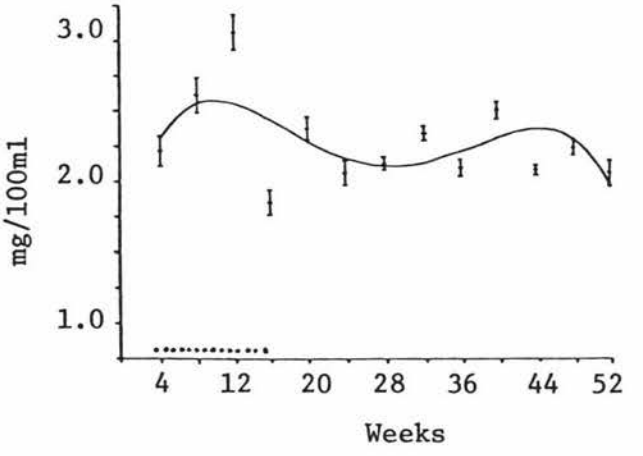


Figure IV: 155

Massey No. 1 dairy unit
Spring calving group
Magnesium
Time in 4 week intervals

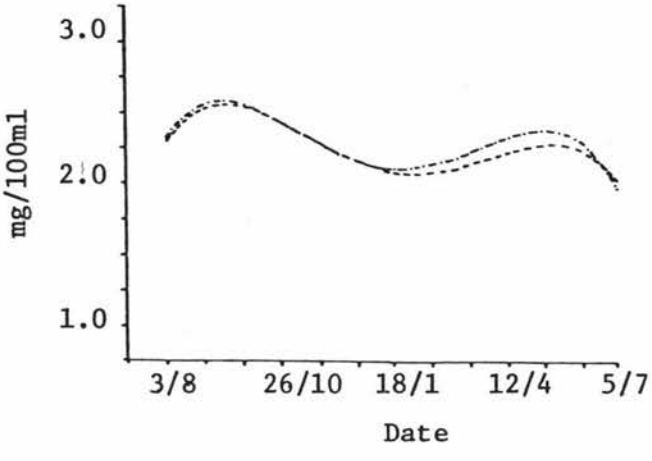


Figure IV: 156

Massey No. 1 dairy unit
Autumn & spring calving groups
Magnesium
Time in 4 week intervals
Simultaneous plot

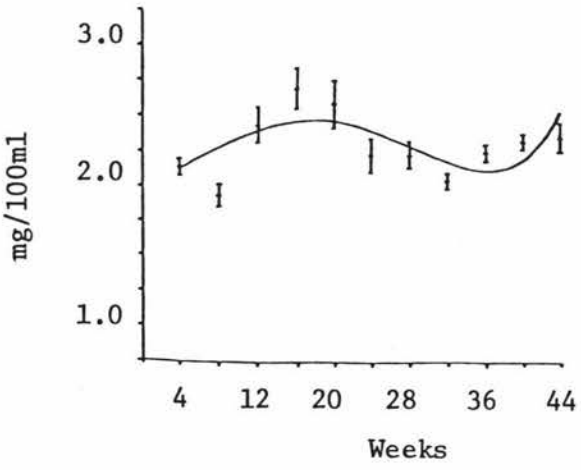


Figure IV: 157

Massey No. 1 dairy unit
Autumn calving group
Magnesium
Weeks in milk

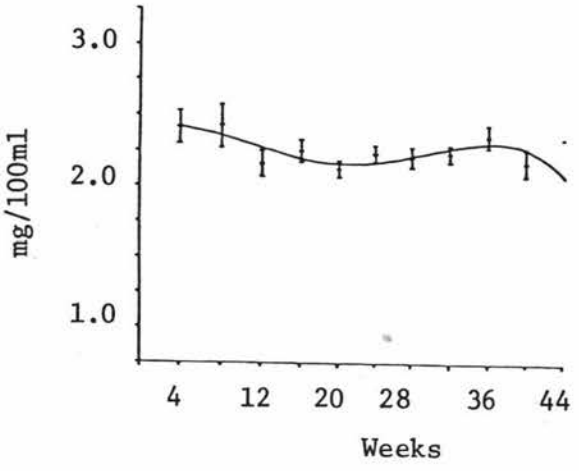


Figure IV: 158

Massey No. 1 dairy unit
Spring calving group
Magnesium
Weeks in milk

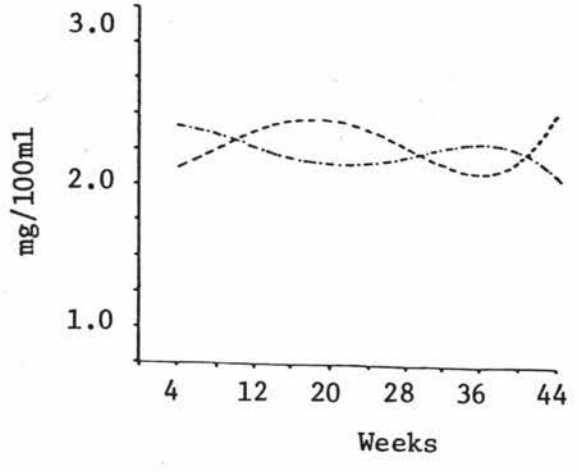


Figure IV: 159

Massey No. 1 dairy unit
Autumn & spring calving groups
Magnesium
Weeks in milk
Simultaneous plot

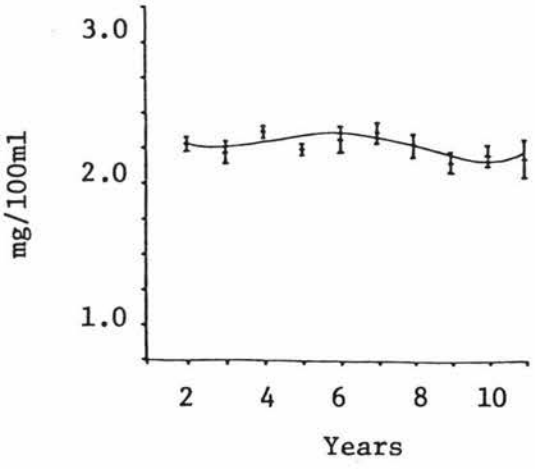


Figure IV: 160

Massey No. 1 dairy unit
Magnesium
Age

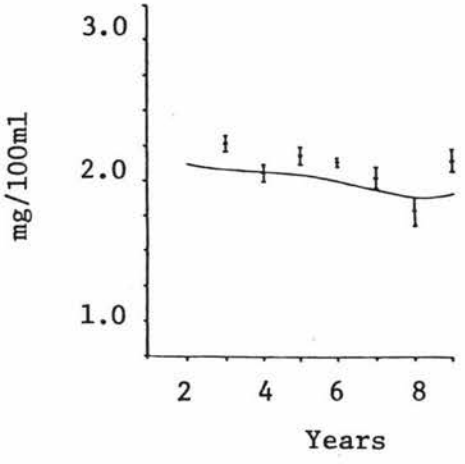


Figure IV: 161

Massey No. 2 dairy unit
Magnesium
Age

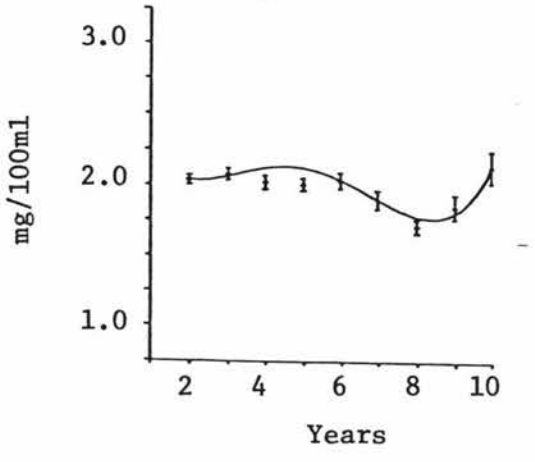


Figure IV: 162

Massey No. 3 dairy unit
Magnesium
Age

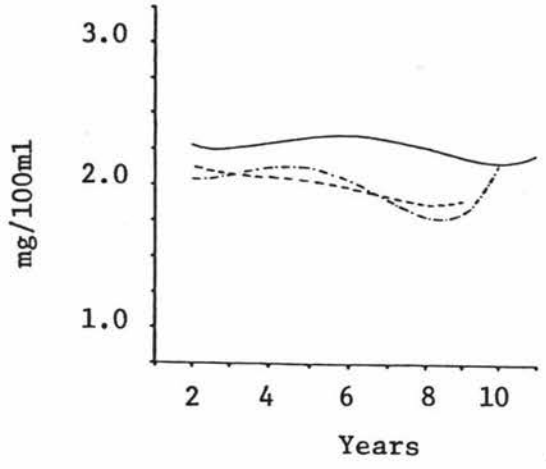


Figure IV: 163

All 3 dairy units Massey
Magnesium
Age

Calcium

The means for serum calcium for the three Massey units differed from each other and from the U.K. mean (Table IV:1). Reference to reports from other workers (see Review of Literature) reveals a still wider range of mean values. Some of the differences reported could be due to differences in the method of sampling, handling and analysis of the material under investigation. The same testing method however was used for both the work of Rowlands *et al.* (1974) quoted in Table IV:1 and for the Massey investigations.

Dietary variations associated with different soil types may provide one explanation for the differences observed. The mean serum calcium levels for Units 1 & 2 were similar and they shared the same soil type. Unit 3 on the other hand had a markedly different soil type and a value for serum calcium that differed considerably from that on Units 1 & 2.

A further explanation for the difference between units could be associated with breed. Unit 1 cattle and the majority of the cattle on Unit 2 were Friesians whereas the cows on Unit 3 were all of the Jersey breed. Breed differences have been recorded (Kitchenham & Rowlands, 1976 ; Rowlands *et al.*, 1977a) although the Jersey was not one of the breeds tested.

The standard deviations for the mean serum values in the Massey herds were also high compared to that reported from the U.K. (Table IV:1). There are three probable explanations for this finding: the number of cows under test in each unit was small relative to the total numbers of cows in the U.K. data (Table IV:1); greater variation in the calcium content of the diet under an all grass grazing situation (such a variation has been reported - see Coggins & Field, 1976; Belyea *et al.*, 1976); and the relatively high experimental error associated with estimations for serum calcium by the methods used (Rowlands & Pocock, 1971).

The graphs for changes due to time of year for the Massey herds (Figs. IV:164-167) showed a low or falling level of calcium in the early spring, a rise during the time when there was a high level of available feed, a plateau in summer and possibly a slight fall in winter. As the amount of variation explained by time of year was greater than that explained by lactation (Table IV:2) the response seen is probably due to changes in the calcium content of the feed. Changes in the calcium levels of the herbage associated with maturity and with changing composition could account for this; for example there is a rise in the clover content of the sward during the spring period (Johns, 1955) and clovers relative to grasses are high in calcium (Beeson and Perry, 1975).

The dominating effect of season was apparent in the plots for calcium against weeks in milk for the separate autumn and spring calving groups on Unit 1 (Figs. IV:175-177). At the same stage of lactation, the striking feature was the rise at 24 and 37 weeks for the spring and autumn calving groups respectively. This was equivalent to the peak which occurred at 28 weeks in the plot of calcium against weeks from the start for this unit (Fig. IV:174).

Falls in serum calcium levels during lactation, particularly over the early periods of high milk production, were noted in the review of literature. This change was not particularly marked in the Massey investigation relative to that due to season as can be seen from the separate spring and autumn calving groups on Unit 1 (Figs. IV:172-174); even though the two groups were at quite different levels of lactation, the curves were remarkably similar. Lactational effects did play some part however, and probably caused each group to be lower than the other during the autumn and spring calving periods respectively.

Although the graphs for the influence of age on serum calcium (Figs. IV:178-181) did not convincingly decrease as the cattle became older (a feature noted in the Review of Literature), there was some evidence for an age effect on Units 2 and 3

although in opposite directions and to a less obvious extent on Unit 1. It should be noted that there were relatively few cattle over nine years old on all these units, thus reducing the chances of measuring an age effect; it is also possible that under the feeding system practiced in New Zealand, enough calcium would be absorbed from the intestine so that variation in bone solubility with advancing age would have only a relatively small effect on serum levels of this element.

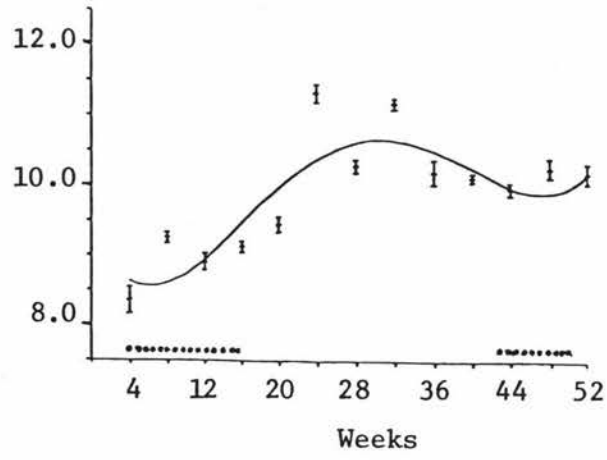


Figure IV: 164

Massey No. 1 dairy unit
Calcium
Time in 4 week intervals

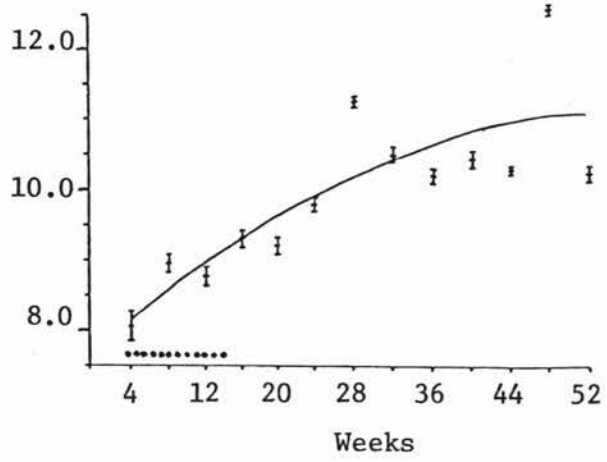


Figure IV: 165

Massey No. 2 dairy unit
Calcium
Time in 4 week intervals

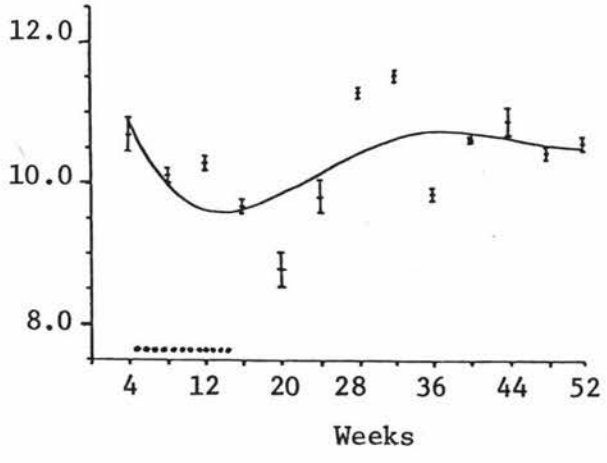


Figure IV: 166

Massey No. 3 dairy unit
Calcium
Time in 4 week intervals

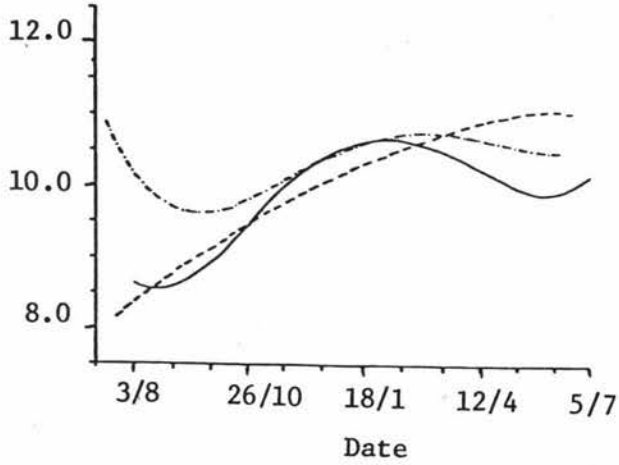


Figure IV: 167

All 3 dairy units Massey
Calcium
Time in 4 week intervals

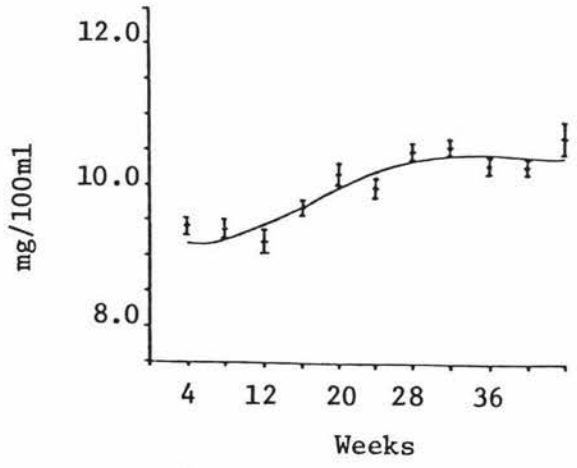


Figure IV: 168

Massey No. 1 dairy unit
Calcium
Weeks in milk

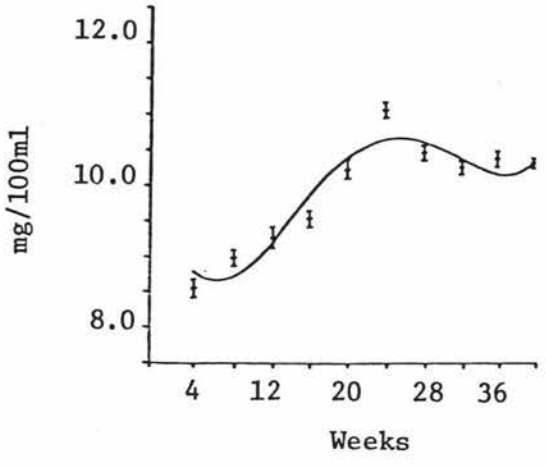


Figure IV: 169

Massey No. 2 dairy unit
Calcium
Weeks in milk

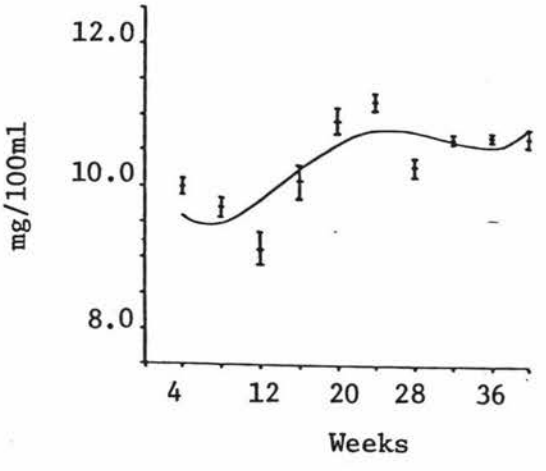


Figure IV: 170

Massey No. 3 dairy unit
Calcium
Weeks in milk

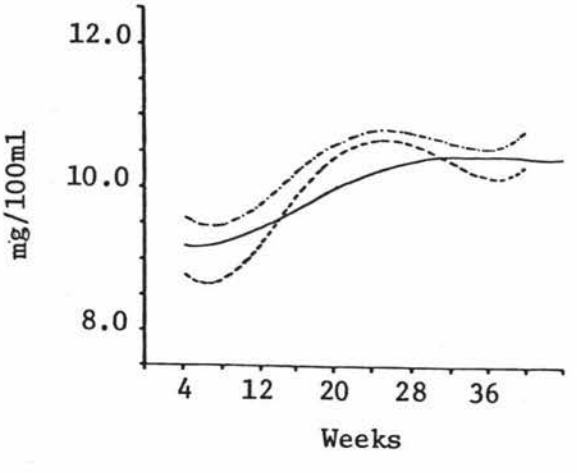


Figure IV: 171

All 3 dairy units Massey
Calcium
Weeks in milk

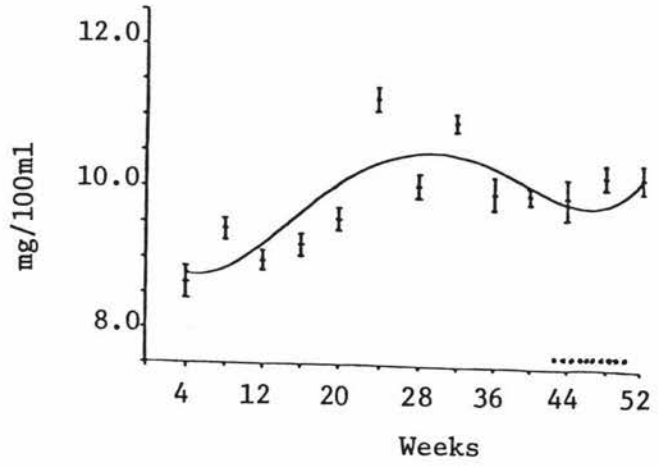


Figure IV: 172

Massey No. 1 dairy unit
Autumn calving group
Calcium
Time in 4 week intervals

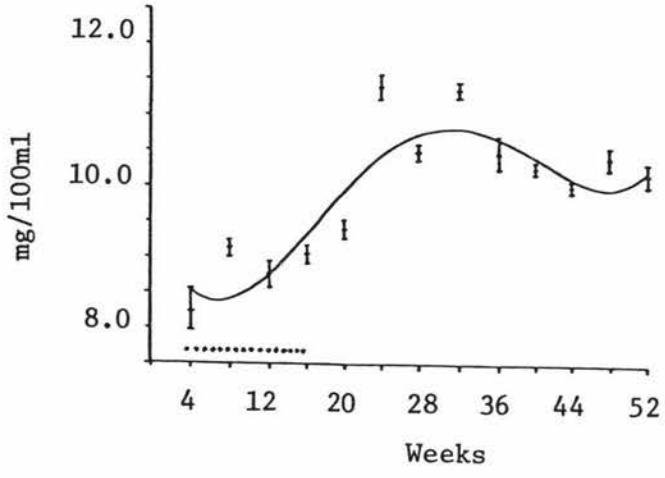


Figure IV: 173

Massey No. 1 dairy unit
Spring calving groups
Calcium
Time in 4 week intervals

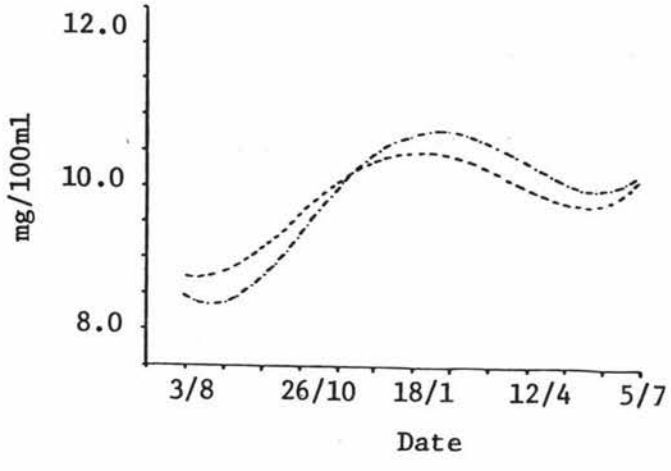


Figure IV: 174

Massey No. 1 dairy unit
Autumn & spring calving groups
Calcium
Time in 4 week intervals
Simultaneous plot

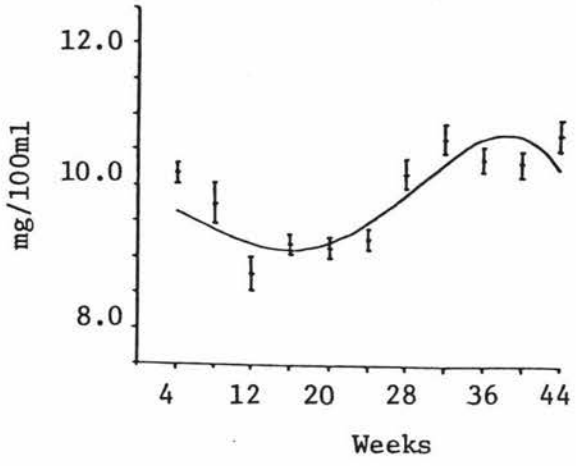


Figure IV: 175

Massey No. 1 dairy unit
Autumn calving group
Calcium
Weeks in milk

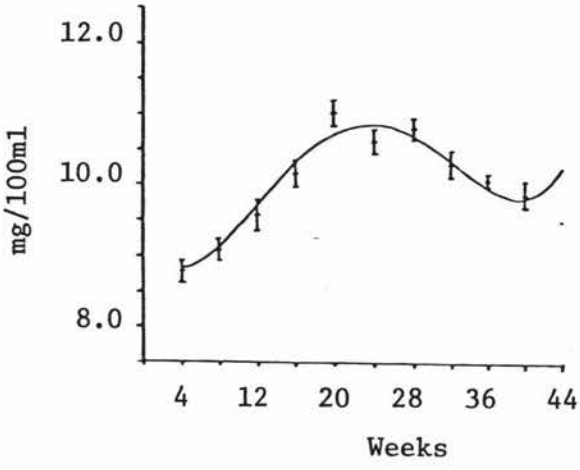


Figure IV: 176

Massey No. 1 dairy unit
Spring calving group
Calcium
Weeks in milk

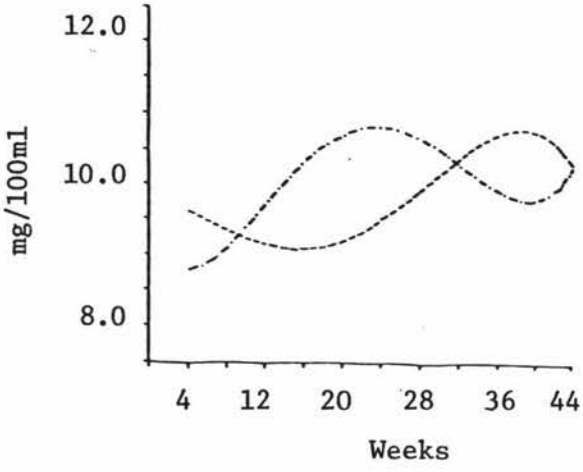


Figure IV: 177

Massey No. 1 dairy unit
Autumn & spring calving group
Calcium
Weeks in milk
Simultaneous plot

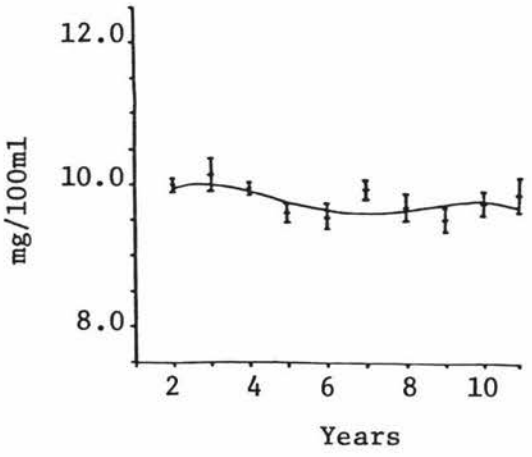


Figure IV: 178

Massey No. 1 dairy unit
Calcium
Age

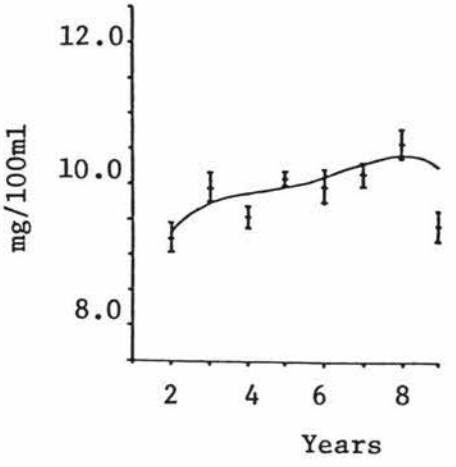


Figure IV: 179

Massey No. 2 dairy unit
Calcium
Age

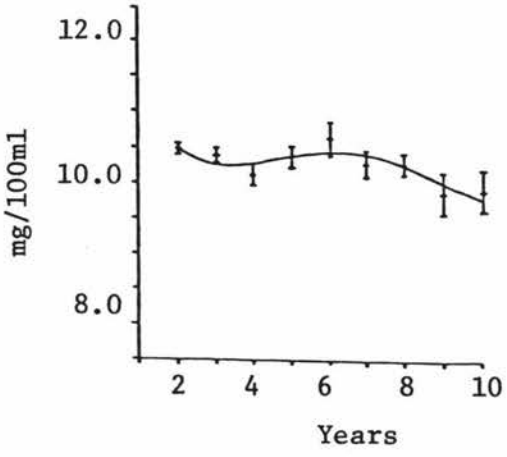


Figure IV: 180

Massey No. 3 dairy unit
Calcium
Age

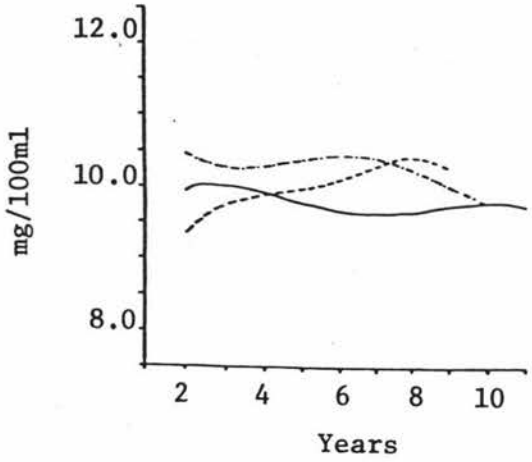


Figure IV: 181

All 3 dairy units Massey
Calcium
Age

Inorganic Phosphate

The values for inorganic phosphate for the three Massey units did not differ markedly from those for the U.K. (Table IV:1) although the standard deviations of the means were larger. Variations in phosphate levels in the diet provide the key to this. The phosphate content of pasture is demonstratively lower in winter than in the summer and autumn (Bisschop, 1964); during this time many cattle in the U.K. are indoors receiving supplementary feed the phosphate level of which has probably been adjusted to exceed the minimum requirements. In New Zealand cattle generally receive supplementary feed only in the form of stored grass as hay or silage and this usually during the late autumn through to early spring period. Phosphate additives are not offered. Thus there is likely to be a wider variation in the quantity and content of phosphate in the diet of the New Zealand cow compared with that offered to her counterpart in the U.K. accounting for the relatively high standard deviation observed in the pooled New Zealand data.

In view of the conflicting reports on the influence of various factors on serum phosphate levels recorded in the literature, (see Literature Review), interpretation of changes in serum inorganic phosphate level and their causation often cannot be made. There appears to be a consensus of opinion however, that serum level is a moderately sensitive indicator of balance of input, internal control and output of this element.

When the graphs were examined for changes in serum phosphate levels with time of year (Figs. IV:182-185) there were iregularities in the curve but all three units illustrated a peak level during the February/April period (late summer-autumn). Should the same changes occur in pasture in New Zealand as were demonstrated by Bisschop (1964) there would be a higher phosphate intake at this time. Shirley *et al.* (1967) similarly has reported a peak serum level of inorganic phosphate in the northern hemisphere autumn (September) while

Payne *et al.* (1974) recorded higher values in the autumn than in summer. All this evidence suggests that changes in serum inorganic phosphate levels with time of year simply reflect natural alterations in the phosphate content of the diet. Why high levels for this element were recorded on Unit 3 (Fig. IV:184) during winter, when both pasture and serum levels should be low, cannot be explained.

The graphs for stage of lactation (Figs. IV:186-189) revealed similar changes for each herd. In all cases there was a rise from a low point at the start of lactation to a high point at or near the end of lactation. While this is likely to be a reflection of the seasonal change in pasture content of phosphate in a seasonally calving herd (Units 2 and 3) it does not explain the situation on Unit 1 where a number of the autumn calving group calved when the pasture phosphate levels were high and then went into a period of relatively low phosphate content in the diet.

Lactation has been reported to result in lower serum inorganic phosphate levels (Payne and Leech, 1964) especially at the time of peak production (Rowlands *et al.*, 1974). Depression in phosphate levels at the time of parturition have also been noted (Sellers & Roepke, 1951). The movement of phosphate into the milk and the failure of parathyroid hormone to adequately mobilise the bone reservoir of phosphate in early lactation could explain these effects (Mayer *et al.*, 1966a, 1968). The changes in phosphate levels in spring and autumn calving groups on Unit 1 (Fig. IV:190-192) illustrate these demands of lactation with the spring calving group being lower than the autumn calving group at and after calving, due to the demands of lactation, and the autumn calving group high during the autumn because the majority ceased lactation and because pasture levels were high at that time. The curve for the autumn calving group fell to a lower winter level as the greater proportion of them calved and commenced lactation.

The change for inorganic phosphate in the spring and autumn calving groups on Unit 1 with lactation stage (Figs. IV:193-195) was dominated by a low winter and spring and high autumn serum levels. However in these plots level of phosphate appears to rise throughout lactation.

With increasing age there was a fall in the level of serum inorganic phosphate on all three Massey units (Figs. IV:196-199). This has already been fully documented (see Review of Literature); the fall of approximately 1.0 mg/100ml from maturity to 8 years of age is believed to be due to reduced bone solubility of this element.

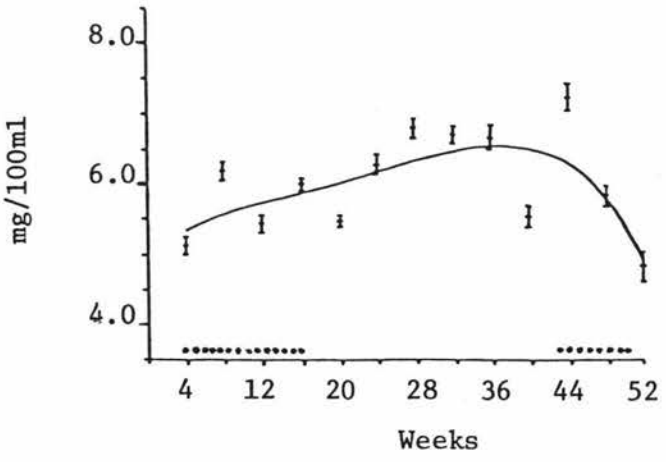


Figure IV: 182

Massey No. 1 dairy unit
Inorganic phosphate
Time in 4 week intervals

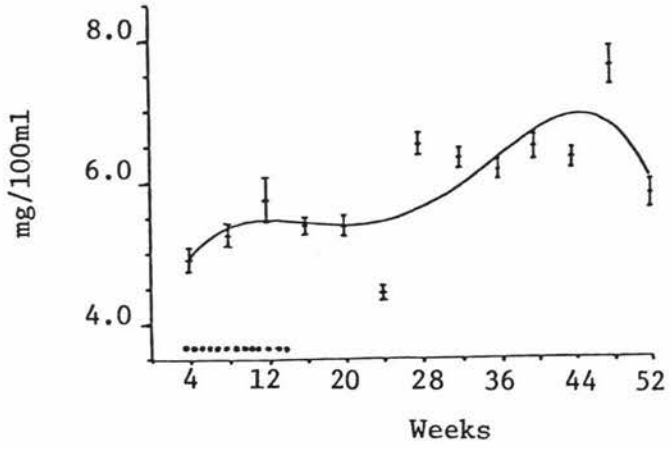


Figure IV: 183

Massey No. 2 dairy unit
Inorganic phosphate
Time in 4 week intervals

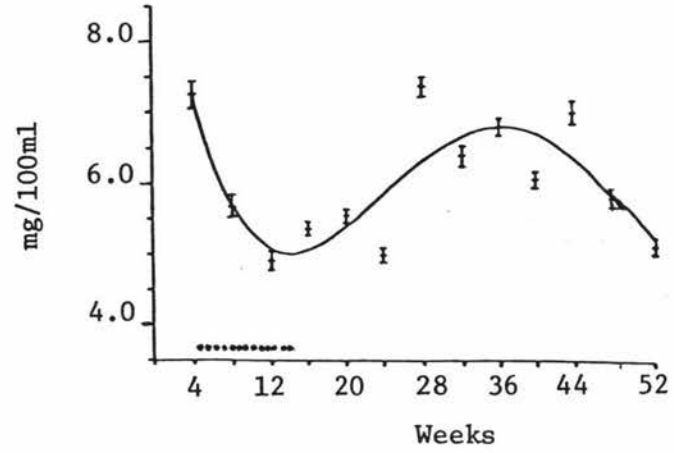


Figure IV: 184

Massey No. 3 dairy farm
Inorganic phosphate
Time in 4 week intervals

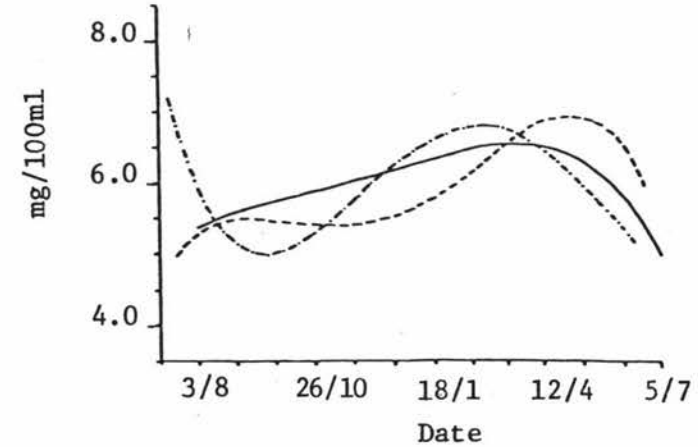


Figure IV: 185

All 3 dairy units Massey
Inorganic phosphate
Simultaneous plot

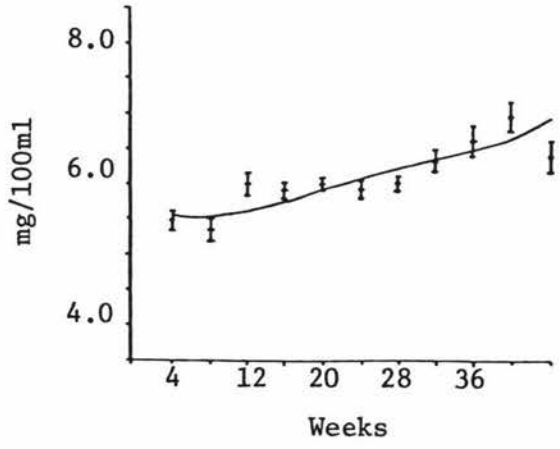


Figure IV: 186

Massey No. 1 dairy unit
Inorganic Phosphate
Weeks in milk

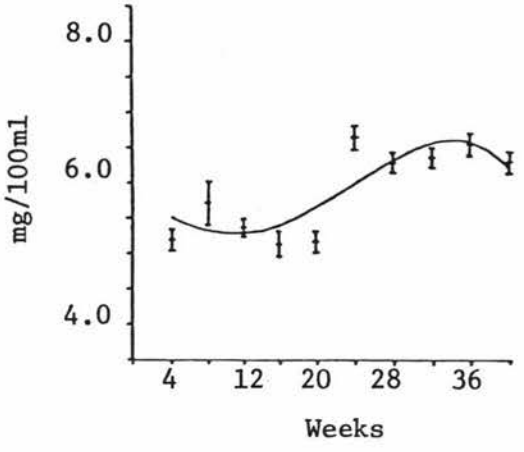


Figure IV: 187

Massey No. 2 dairy unit
Inorganic Phosphate
Weeks in milk

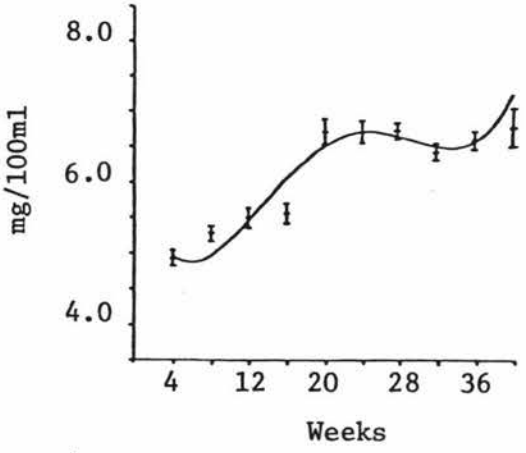


Figure IV: 188

Massey No. 3 dairy unit
Inorganic Phosphate
Weeks in milk

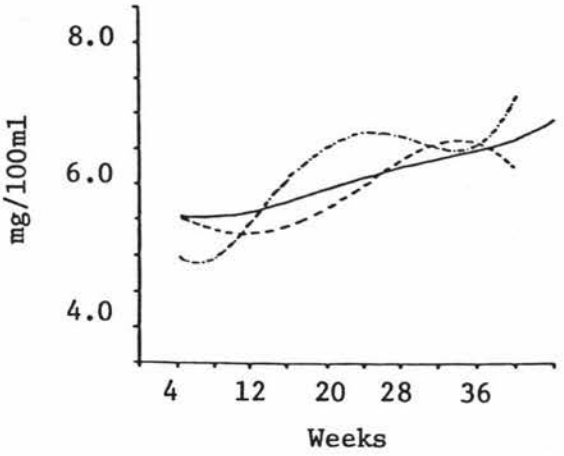


Figure IV: 189

All 3 dairy units Massey
Inorganic Phosphate
Weeks in milk

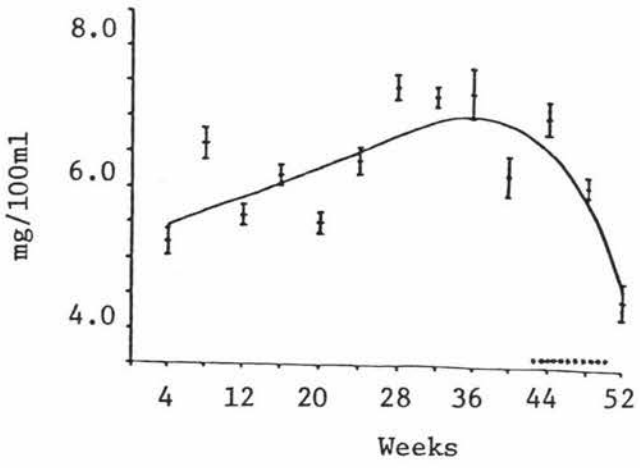


Figure IV: 190

Massey No. 1 dairy unit
Autumn calving group
Inorganic phosphate
Time in 4 week intervals

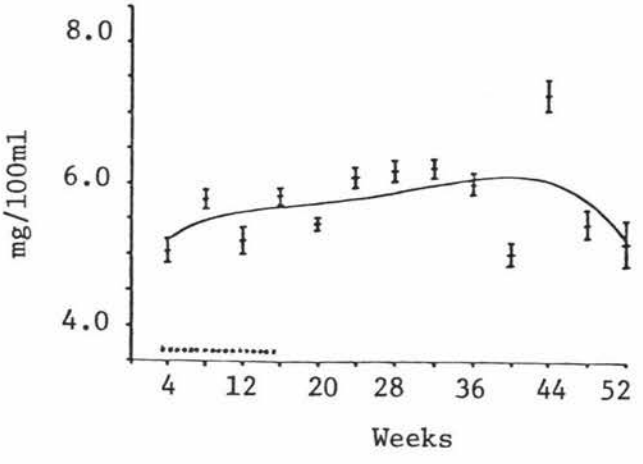


Figure IV: 191

Massey No. 1 dairy unit
Spring calving group
Inorganic phosphate
Time in 4 week intervals

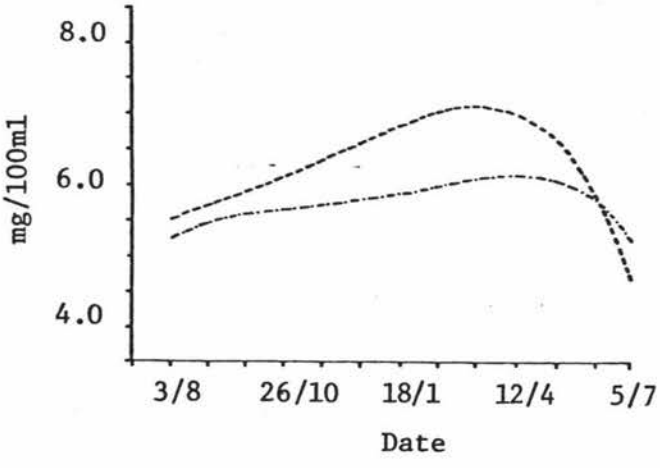


Figure IV: 192

Massey No. 1 dairy unit
Autumn & spring calving groups
Inorganic phosphate
Time in 4 week intervals
Simultaneous plot

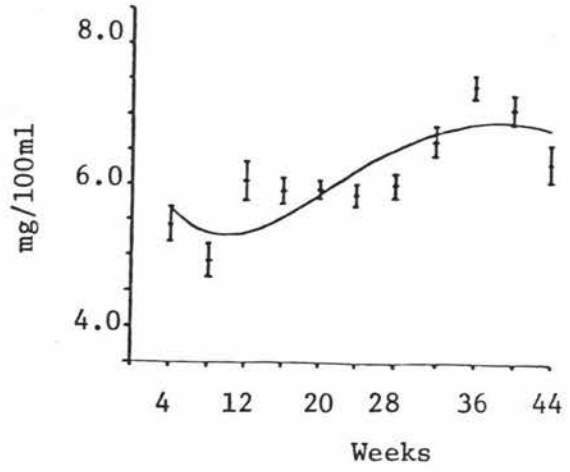


Figure IV: 193

Massey No. 1 dairy unit
Autumn calving group
Inorganic phosphate
Weeks in milk

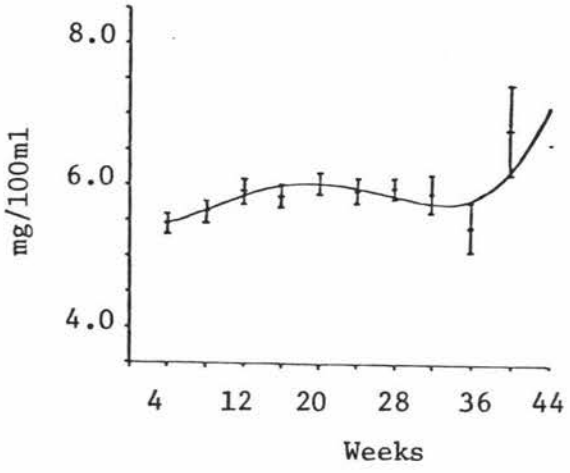


Figure IV: 194

Massey No. 1 dairy unit
Spring calving group
Inorganic phosphate
Weeks in milk

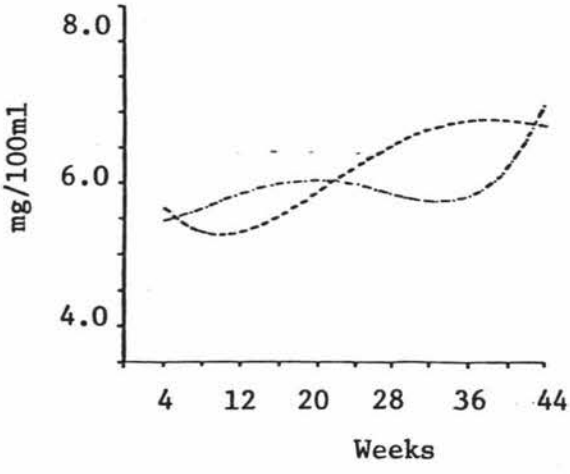


Figure IV: 195

Massey No. 1 dairy unit
Autumn & spring calving groups
Inorganic phosphate
Weeks in milk
Simultaneous plot

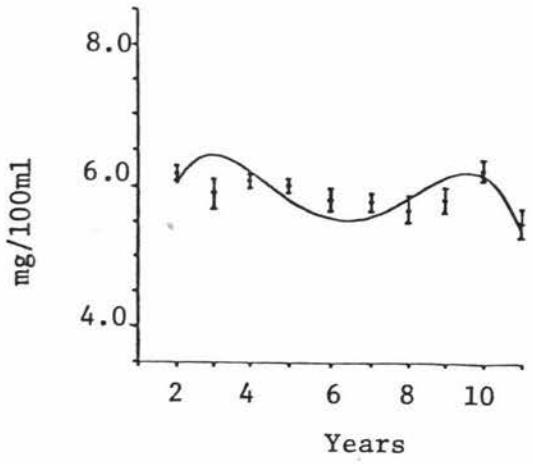


Figure IV: 196

Massey No. 1 dairy unit
Inorganic Phosphate
Age

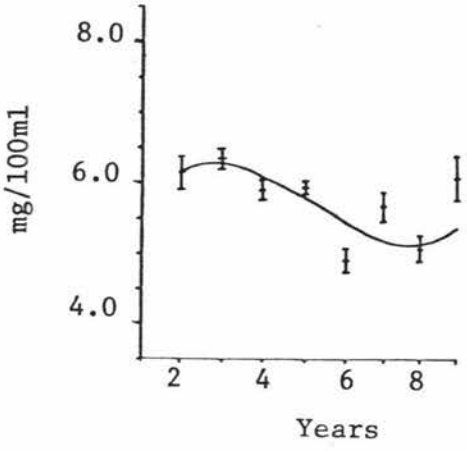


Figure IV: 197

Massey No. 2 dairy unit
Inorganic Phosphate
Age

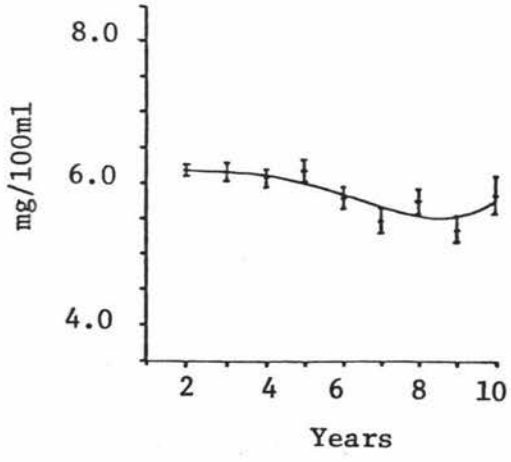


Figure IV: 198

Massey No. 3 dairy unit
Inorganic Phosphate
Age

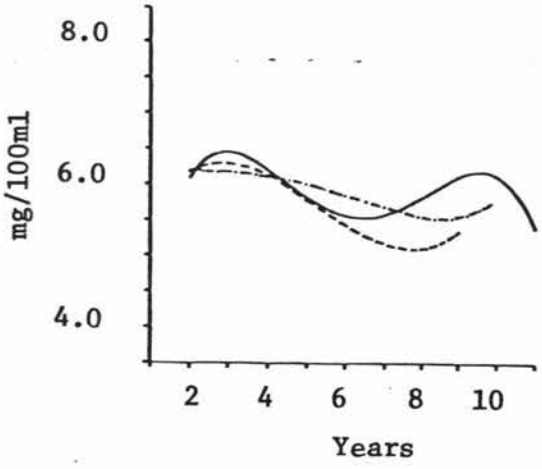


Figure IV: 199

All 3 dairy units Massey
Inorganic Phosphate
Age